Characterization of adaptation of a spring *Brassica napus* doubled haploid population derived from a winter by spring cross

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Abstract

Genetic diversity available in the Western Canadian adapted *Brassica napus* germplasm has decreased substantially in recent years, leading plant breeders to search for new sources of diversity. This study aims to determine whether the relevant agronomic traits as well as increased sub-zero temperature tolerance can be combined within the context of a European winter-type by Australian spring-type *Brassica napus* doubled-haploid (DH) population containing 115 DH lines. The hypothesis is that DH individuals will be found that possess sub-zero temperature tolerance as well as early flowering and maturity. There are three major objectives in this research: 1) characterization of the agronomic traits of this population including flowering times and maturity using field experiments, 2) evaluation of sub-zero temperature tolerance using field experiments, and 3) evaluation of low temperature germination ability using laboratory experiments. Analysis of the agronomic, sub-zero temperature tolerance and germination data together suggests that, since there are examples in this particular population, it is possible to combine increased sub-zero temperature tolerance with early maturity in a spring-like growth habit. The combination of increased sub-zero temperature tolerance, lack of vernalization requirement and early maturity in an otherwise winter-type genetic background represents another step in germplasm development within *Brassica napus*.

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List of Abbreviations Used

DH	Doubled haploid
USDA	United States Department of Agriculture
lb/ac	Pounds per acre
ANOVA	Analysis of variance
LSD	Least significant difference
RCBD	Randomized complete block design
GLM	General linear model
CMS	Cytoplasmic male sterility
F1	First filial generation
QTL	Quantitative trait loci

1. Introduction

Over the last number of years, high selection intensities for a variety of breeding objectives such as high oil content, herbicide tolerance, disease resistance and higher yield have resulted in a decrease in the amount of genetic variation available to plant breeders in the annual, Canadian-adapted, *Brassica napus* germplasm. Concern has been raised over the potential to continually improve this germplasm as a result of this narrowing genetic diversity (Becker et al., 1995; Hasan et al., 2006; Fu and Gugel, 2010; Kebede et al., 2010), especially since most *B. napus* varieties grown commercially today are hybrid cultivars that require genetic diversity between parental lines to achieve high levels of heterosis (Brandle and McVetty, 1989).

Canola quality *B. napus* germplasm is typically separated into three major heterotic pools, each of which is viewed to be genetically distinct from the others. The first group consists of spring germplasm, the second group consists of winter germplasm, and the third group consists of semi-winter or intermediate germplasm. The intermediate types are typically well-adapted to Asian climates and contain varying degrees of the characteristics of both the spring and winter types (Diers and Osborn, 1994). Diers and Osborn were among the first to suggest that these groups present different potential sources for useful genetic diversity when crosses are made between them. High levels of heterosis observed when crossing two genetically distinct breeding lines have led many plant breeders to import non-adapted *B. napus* germplasm from around the world in an effort to increase the genetic diversity found in the annual germplasm pool. The benefits to strategically broadening the genetic base of a *B. napus* breeding program are two-fold. Firstly, increased inbred line performance, and secondly increased hybrid vigour

and yields. A directly applicable example of the heterosis that may be achieved by crossing amongst these groups is increased seed yields within spring hybrids produced with some winter background in one or other of the parents (Butruille et al., 1999; Quijada et al., 2004; Udall et al., 2004). Yield increases delivered using this method, however, are typically inversely proportional to early maturity in *B. napus* (Butruille et al., 1999). Due to the late maturity of winter *B. napus* varieties, it is crucial to select for spring-like, early maturing lines derived from crosses made between winter and spring parents, while attempting to maintain the winter background. In this way, the selected progeny of the cross will be compatible with the environment present in Western Canada. When increasing the diversity of *B. napus* breeding populations, a balance must be found between un-adapted diverse lines and adapted but less diverse lines.

Like many cultivated crop species, *B. napus* canola tends to yield higher in regions where longer growth seasons allow for later maturity. In the Canadian prairies, a relatively short growing season precludes using high-yielding, but relatively late-maturing germplasm in a commercial breeding program. As such, it is difficult to utilize winter germplasm for spring breeding line improvement, as lateness of flowering and later maturity are typically introgressed from the winter parent into the progeny when crossing winter by spring lines (Rahman, 2011). This problem could be somewhat mitigated, however, by breeding for increased levels of sub-zero temperature tolerance. A potential increase in cold tolerance conferred to the progeny of winter by spring crosses may allow for the crop to be seeded earlier in the spring. In this way, a longer growing season could be harnessed to increase yield. Alternatively, hybrids could be seeded in regions that were previously considered inhospitable to *B. napus*. Additionally, the winter germplasm derived from a genetically distinct heterotic pool may carry with it other traits of importance such as disease resistance. Heterosis may be achieved when creating hybrids, in

which one parent contains a winter genetic background, even though that line may not exhibit a winter-like phenotype. As a result, it is important to understand a breeder's ability to harness the winter germplasm as a source for germplasm diversity, including traits such as sub-zero temperature tolerance.

Most plants adapted to temperate climates undergo temperature acclimation when exposed to increasingly cold temperatures. At some point exposure to sub-zero temperatures will result in cell death, usually due to a rupture of the plant cell membrane caused by severe dehydration (Steponkus, 1984). Germination is delayed or is reduced at temperatures below 10 °C (Nykiforuk and Johnson-Flanagan, 1999) and typically does not occur at temperatures lower than 2 °C (Nykiforuk and Johnson-Flanagan, 1994 and 1997). The lowest temperature at which seedling growth of *B. napus* occurs is approximately 5 °C (Morrison et al., 1989).

Vernalization is often associated with tolerance to sub-zero temperatures because of the requirement for vernalization in winter *B. napus* germplasm. The doubled haploid population used for the research described here was derived from a single cross between a spring *B. napus* cultivar of Australian background and a winter *B. napus* cultivar of European background containing a quantitative vernalization requirement. The vernalization trait was expected to segregate such that some doubled haploid (DH) individuals would require vernalization and some would not. DH individuals from this population that took an extended time to flower and mature, and thus seemed to require vernalization, were discarded due to their irrelevance in a commercial breeding program and the impossibility of evaluation in a Western Canadian field experiment. In the context of the current research, the vernalization requirement can be considered irrelevant as it has been demonstrated that tolerance to sub-zero temperatures can be inherited separately from vernalization (Hawkins et al., 2002).

A cross between a European winter-type cultivar, Caracas, and an Australian spring-type cultivar, AG-Outback, was selected for this research on the basis that, by using totally unrelated parents, a diverse population of genetically and phenotypically distinct DH lines would be created. Additionally, the European winter and Australian spring germplasm often carry improved alleles in a number of key traits outside the scope of this research, making them primary candidates for early breeding crosses and field evaluation in a commercial breeding program (Dr. A.D.W. Grombacher, pers. comm.). The major objective of this research was to understand the effectiveness of transferring cold tolerance into spring *B. napus* lines through crosses with winter germplasm, and whether this sub-zero temperature tolerance can be associated with other agronomic traits of interest. The following experimental hypotheses were developed in an effort to answer this broad query:

1. The DH lines derived from this winter x spring cross will show significant variation in a number of agronomic traits including early vigour, days to flower, height and maturity.
2. The DH lines derived from this winter x spring cross will show significantly greater sub-zero temperature tolerance than either the winter or spring parent.
3. The DH lines derived from this winter x spring cross will germinate in cold temperatures significantly better than either the winter or spring parent.
4. Increased sub-zero temperature tolerance and low temperature germination traits can be combined with desirable agronomic traits such as early flowering and maturity.

Three experiments were designed to test these experimental hypotheses. The first experiment was designed to evaluate the agronomic characteristics of the population, the second experiment tested sub-zero temperature tolerance in the field, and the third experiment tested seed

germination at low (near 0 ºC) temperatures. The objectives of these experiments are summarized below:

1. Field agronomic evaluation:
    a. Determine an agronomic profile of each line by evaluating early vigour, days to flower, height and days to maturity.
2. Field tolerance to sub-zero temperatures evaluation:
    a. Determine the sub-zero temperature tolerance of each winter by spring DH line in a field setting using fall seeding dates.
    b. Evaluate the level of chlorosis after major frost events of plants at different growth stages as an indicator of frost tolerance.
3. Low temperature seed germination evaluation:
    a. Determine whether there is a difference in low temperature germination between checks, parental lines, and DH lines of this population.
    b. Evaluate germination percentages of selected DH lines and checks at near freezing, intermediate, and room temperatures.

An important reason for conducting agronomic evaluations of all the DH lines within this research project was to determine whether sub-zero temperature tolerance appears to be linked in any way to a desirable (e.g. early season vigour) or deleterious agronomic characteristic (e.g. late maturity).

2. Literature Review

2.1. Family Brassicaceae

Brassica napus is an allotetraploid plant species belonging to family *Brassicaceae*, which includes cruciferous vegetables such as cabbage, and oilseed crops such as mustard. Within family *Brassicaceae*, the most closely-related species are found within genus *Brassica* and consist of the diploid species *Brassica nigra, Brassica oleracea,* and *Brassica rapa*, as well as the allotetraploid species *Brassica napus*, *Brassica carinata* and *Brassica juncea* (U, 1935). The close genetic relationship between the aforementioned three diploid species allowed them to interbreed, resulting in the three allotetraploid species. Specifically, *B. napus* (2n=38, AACC) is the result of interspecific hybridization between *B. oleracea* L. (2n=18, CC) and *B. rapa* L. (2n=20, AA) followed by chromosome doubling (U, 1935).

2.2. Economic Importance of Canola

Historically known as rapeseed, or oilseed rape, *B. napus* is now colloquially referred to as canola within Western Canada. The term canola (an abbreviated term for Canadian oil – "can-ola" (Canola Council of Canada A, 2016)) also encompasses varieties of many different *Brassica* species, including *B. juncea* and *B. rapa*, that meet a certain set of seed oil and meal quality criteria. According to the most recent data from the United States Department of Agriculture (USDA), rapeseed accounted for approximately 13.5 % of global oilseed production in 2015/16, making it the second largest global oilseed crop after soybean (60.0 %; George, 2016). Of the global production in 2015/16, Canada contributed approximately 26.2 % (George, 2016). This

makes Canada the second-largest rapeseed-producing region in the world behind the European Union. Canola production within Canada, the vast majority of which is *B. napus*, continues to grow in economic importance. Additionally, canola oil contains healthier monounsaturated fatty acids, compared to the polyunsaturated fatty acids of other oil crops such as corn and soy (Gray and Malla, 2001). This key difference is now better understood in many markets around the world, further enhancing the importance of canola as a staple export crop within Western Canadian agriculture.

2.3. Breeding Objectives for *Brassica napus*

The primary breeding objectives in Canada for *B. napus* have changed somewhat over time. Originally, rapeseed cultivars contained high levels of erucic acid and glucosinolates, which resulted in an unpleasant and unpalatable bitter taste, rendering those cultivars useful only for industrial lubricant oils. At that time, it was not possible to market rapeseed oil as a food product. The primary breeding objective for rapeseed in Canada throughout the 1960s and 1970s was to produce varieties that yielded seed containing both low glucosinolate content and low erucic acid content in an effort to use rapeseed oil as a food product. The successful combination of these two traits through traditional plant breeding methods resulted in the creation of the term "canola" as part of an effort to distinguish this new product from the pre-existing rapeseed crop. Canola quality *B. napus* seeds must produce oil containing a fatty acid profile with less than 2 % erucic acid, as well as less than 30 micromoles glucosinolates per gram of air-dried, oil-free meal (Canola Council of Canada A, 2016). Varieties that meet these oil and meal criteria may be classified as canola-quality and registered as canola cultivars. Because of this registration requirement, breeding for this specific oil profile remains a highly relevant breeding objective

even in present-day breeding programs. Due to the narrowing of genetic diversity that occurred when originally breeding for double-low oil quality, it is necessary to incorporate wider sources of genetic diversity into present-day breeding programs to continue improving other key traits such as yield and disease resistance (Downey and Rimmer, 1993). Introgressing traits of interest from materials of wide genetic diversity can result in oil profiles that do not meet the canola quality standard. Because of this, crossing using diverse germplasm and screening for candidate lines that are well within the canola quality standard remains a significant characteristic of commercial canola breeding programs today.

In addition to seed oil quality, a number of other breeding objectives have been pursued since the development of double-low canola. Incorporating new mechanisms of resistance to fungal pathogens such as blackleg, sclerotinia, and clubroot continues to be an important focus as these pathogen populations are constantly shifting and defeating existing resistance mechanisms. Due primarily to the advent of the Roundup Ready, Clearfield and Liberty Link herbicide tolerance systems, there is currently a high market demand for herbicide tolerant cultivars. A major breeding objective for canola, especially when viewed from a commercialization perspective, continues to be incorporation of resistance to a major herbicide such as glyphosate, glufosinate or imidazolinone. While attempting to maintain and improve the traits noted above, improvement to yield is always a high-priority breeding objective and continues to be the trait of supreme importance. Additional traits of interest include abiotic stress tolerance, improvements and/or modifications to fatty acid profile, pest resistance, larger seed size, and higher yielding inbred lines and hybrid varieties (Canola Council of Canada B, 2016).

2.4. Heterotic Groups

Brassica napus germplasm is separated into three major groups, each of which is genetically distinct. The first group consists of spring germplasm, the second group winter germplasm, and the third group semi-winter or intermediate types that contain variations of both the spring and winter types (Diers and Osborn, 1994). Spring-type varieties are typically grown in North America and Australia, winter varieties in Europe, and semi-winter varieties in areas of Asia. Since these three groups have been bred and adapted for different regions of the world, allelic variation exists between groups. For example, winter and semi-winter varieties often contain different disease resistance alleles than do spring types due to the race-specific avirulence genes present in their geographic area of adaptation (Rouxel et al., 2003). Different alleles for other traits of interest could be found between these groups, because of genetic variation due to adaptation. When looking to increase the genetic variation of a breeding population, it is often efficient to explore the most easily-introgressed sources of genetic diversity first, rather than looking to exotic landraces or related species. In the case of *Brassica napus*, this means the winter and semi-winter heterotic pools. In this scenario, it is also unlikely that *in vitro* techniques will be necessary to facilitate successful crossing as it might be in the case of exotic landraces or other species.

High selection intensities for a variety of breeding objectives have resulted in concern over the narrowing of genetic diversity in the spring form of *B. napus* in recent years (Becker et al., 1995; Hasan et al., 2006). There may be presently little genetic variation remaining in the well-adapted germplasm for many of the aforementioned traits (Fu and Gugel, 2010). Concern has arisen that continued improvement in *B. napus* varieties may not be possible without increasing genetic diversity in breeding programs first (Kebede et al., 2010). Since *B. napus*

exhibits significant heterosis when two genetically distant lines are crossed together (Udall et al., 2004), plant breeders have been looking to utilize the untapped genetic diversity of germplasm from other geographies to the fullest extent possible. Utilizing the other heterotic groups as a genetic resource to widen the diversity of the spring group would lessen the problem of narrowing diversity when breeding for spring types. However, the pace of yield improvements and existing oil quality standards must be maintained throughout efforts to increase diversity.

2.4.1. Introgression of Genetic Diversity from Winter to Spring

Because of the genetic distance and allelic differences between spring and winter types, Diers and Osborn (1994) suggest that the winter germplasm likely presents a strong source for introgressing genetic diversity into the spring-type breeding programs. This may result in increased seed yields among hybrids produced with one parent containing some winter background (Quijada et al., 2004). Additionally, there has been a demonstrated and commercially-deployed increase in seed yield among spring *B. napus* open-pollinated varieties when the genetic diversity of winter types has been utilized during development (Kebede et al., 2010 and Dr. A.D.W. Grombacher, pers. comm.). This confirms that winter germplasm can be used successfully to increase heterosis and yield in both open-pollinated and hybrid spring growth habit *B. napus*.

There are a number of challenges to overcome in the practical use of progeny from a winter x spring cross. The primary challenge is the later maturity of many breeding materials containing winter genetics, as lateness of flowering and late maturity are typically traits that are introgressed from the winter parent when crossing winter by spring types (Rahman et al., 2011). In Saskatchewan, growing season length precludes using such long-season, late maturing

germplasm in a breeding program. Yield increases are typically inversely proportional to early maturity. The longer a plant has to branch out, produce more flowers and set seed successfully, the more seed it can theoretically produce. The correlation between yield increase and late maturity was demonstrated by Butruille et al. (1999) in the specific case of introgressing winter germplasm into spring *B. napus* material. This is the major hurdle to successfully utilizing the winter germplasm for improvement of spring *B. napus*.

When grown in the Western Canadian prairies, many *B. napus* inbred lines containing some amount of winter background also exhibit a plant architecture that may not be acceptable. For example, a taller growth habit and more focus on vegetative biomass production are prevalent characteristics. A more desirable plant architecture would include dense branching and longer silique structure with a wider podding zone. This architecture is more conducive to proper swathing and maximum seed production, but is not commonly found in germplasm with a winter background. When creating hybrids, as is done in most *B. napus* breeding programs, the phenotype of winter germplasm may be mitigated by crossing to other parental lines with a more acceptable plant architecture.

In addition to the demonstrated increase in yield in the progeny of winter by spring crosses, Kole et al. (2002) demonstrated that an increase in sub-zero temperature tolerance was also likely to be transferred into the spring material. The winter germplasm has been bred for surviving low temperatures, since the environment they grow in sometimes reaches 0 ºC or lower during the overwintering period (Rapacz and Markowski, 1999). Conversely, spring-type lines have not been bred for survival in low temperatures because they are not exposed to that type of environment in their growth cycle (Rapacz and Markowski, 1999). Rapacz and Markowski (1999) identified the difference in level of sub-zero temperature resistance between winter and

spring *B. napus* and concludes that spring forms only seem less tolerant towards freezing temperatures. In fact, winter inbred lines derived from winter germplasm crossed to spring material showed increased levels of winter survivability, due possibly to genetic variation for increased tolerance being transferred from the spring germplasm (Rapacz and Markowski, 1999).

2.5. Cold Tolerance and Vernalization

Cold tolerance and freezing tolerance are two terms that are used throughout the literature to describe the ability of a plant to withstand cold and sub-freezing temperatures. "Freezing tolerance" and/or "sub-zero temperature tolerance" most appropriately refer to an increased survivability when exposed to temperatures below 0 ºC (Kole et al., 2002), usually derived from changes to the cell membrane lipid composition (Thomashow, 1998). Freezing tolerance can be derived from cold acclimation processes or simply be inherent to the genetic makeup of the plant (Teutonico et al., 1995). Cold tolerance is used to describe the temperature range between approximately 10 ºC and 0 ºC at which cold acclimation occurs, as well as temperatures below 0 ºC. When some plant species are exposed to increasingly cold temperatures, they undergo an adaptive process known as cold acclimation in which changes to gene expression are made to increase tolerance to the lower temperatures (Shinozaki and Yamaguchi-Shinozaki, 1996). These inherent cell processes, mediated by differential gene expression, confer increased tolerance to sub-zero temperatures upon a canola plant (McClinchey and Kott, 2007). As such, specific allele combinations inherent to the winter germplasm may confer increased sub-zero temperature tolerance within a spring *B. napus* (Kole et al., 2002).

When exposed to temperatures below freezing, the primary site of injury in the plant is the cell membrane, which may rupture due to severe dehydration (Steponkus, 1984). Cold-

regulated genes such as *Bn*COR25, which code for dehydrin-like proteins, have been suggested as one explanation as to how plants are able to protect against the dehydration associated with lethality at low temperature (Chen et al., 2011). Additionally, the *B. napus* gene *BN115* appears to function in a similar manner whereby a protein product is targeted to the chloroplast membrane to confer resistance to the membrane damage that results from freezing (Sangwan et al., 2001). Sangwan et al. (2001) also identified how transcription of *BN115* appears to be regulated by membrane rigidification, cyto-skeletal reorganization, and action of a variety of protein kinases. These responses potentially play a role in activating sub-zero temperature tolerance in winter *B. napus* germplasm. High expression levels of *Bn*CBF (C-repeat/dehydration-responsive element binding factor) genes in response to cold conditions will activate a cold-response pathway leading to increased expression of *Bn*COR genes (Savitch et al. 2005; and Jaglo et al. 2001). Phenotypically, the overexpression of *Bn*CBF15 and *Bn*CBF17 resulted in dwarf plants with thicker, waxier leaves that required a longer timeframe to bolt and flower (Savitch et al., 2005). The functional amino acid sequences of CBF proteins are highly conserved in all flowering plants, not just *B. napus* or those species that typically cold acclimate (Jaglo et al., 2001). Other genes, such as *hsp90* belonging to the heat-shock protein family or stress protein family in *B. napus* have also been identified as playing a role in the physiological cold acclimation process by aiding in transport of polypeptides that may protect against dehydration, or perhaps even playing a stabilization role themselves (Krishna et al., 1995). Nonetheless, the protein product of *hsp90* has been shown to accumulate, especially in juvenile tissues, under cold conditions (Krishna et al., 1995). Genes within these families are candidates for allelic comparisons between winter and spring varieties, to determine if any of them play a role in cold tolerance within the winter germplasm. While beyond the scope of this work, it is

important to understand that many genes and intragenic interactions play a key role in mediating the response to cold acclimation and sub-zero temperature tolerance.

2.5.1. Quantitative Vernalization Requirement

Winter *B. napus* varieties must undergo vernalization – exposure to an extended period of low, non-freezing temperatures, in order to flower and set seed (Hawkins et al., 2002). The vernalization requirement can range from being a weak (quantitative) requirement or an obligate (qualitative) requirement (Hawkins et al., 2002). A weak vernalization requirement is characterized by a phenotype in which the plant will eventually flower without ever being exposed to the low temperatures that are typically required for proper vernalization to occur (Hawkins et al., 2002). However, if exposed to the vernalization conditions, a plant with a weak vernalization requirement will flower and produce seed more efficiently. In contrast, a plant with an obligate vernalization requirement must be vernalized before reproductive development will occur (Hawkins et al., 2002). The vernalization requirement in *B. napus* is controlled by up to three quantitative trait loci (Ferreira et al., 1995). Photoperiod has also been shown to be an important regulator of both vernalization and sub-zero temperature tolerance (Rapacz and Markowski, 1999).

Both Teutonico et al. (1995) and Ferreira et al. (1995) present data that strongly argue how sub-zero temperature tolerance is controlled by a number of genes within linkage groups that are separate from those linkage groups that contain genes that control vernalization. Hawkins et al. (2002) supports this point, and concluded that sub-zero temperature tolerance and vernalization can be inherited separately in *B. napus*, since separate signals and pathways control the cold tolerance and vernalization responses. In addition, Teutonico et al. (1995) demonstrated

how inherent and acclimated sub-zero temperature responses are separately controlled. While sub-zero temperature tolerance is necessary to survive the conditions necessary for proper vernalization, it is clear that the winter type vernalization requirement is inherited separately from sub-zero temperature tolerance. Indeed, Hawkins et al. (2002) demonstrated that a breeding line derived from a winter by spring cross will exhibit excellent sub-zero temperature tolerance in the absence of a vernalization requirement. This supports the similar conclusions Markowski and Rapacz (1994), which indicated no relationship between sub-zero temperature tolerance and vernalization requirement in winter rapeseed doubled haploid (DH) lines. More work in this area is required, however, as the role of many of the cold response-related genes in *B. napus* remain unexplained at present (Chen et al. 2011).

2.6. Low Temperature Germination Testing

Achieving a high percentage germination of *B. napus* seeds exposed to cold temperatures is important to establishing a proper plant stand and reducing seedling susceptibility to soil borne pathogens (King et al., 1986). In *B. napus,* low temperature germination tests have been thoroughly conducted and generally note that germination is reduced and delayed at temperatures below 10 ºC (Nykiforuk and Johnson-Flanagan, 1999). This supports earlier findings indicating that temperatures below 15 ºC cause delays in germination in *B.napus* (Acharya et al., 1983). At 5 ºC and 7.5 ºC, Acharya et al. (1983) found that less than 12 % of the seeds of two *B. napus* lines germinated after 48 hours, and less than 53 % of the same seeds germinated after 72 hours. Non-homozygous seeds selected for fast germination at 2 ºC (King et al., 1986) and at 10 ºC (Acharya et al., 1983) resulted in the identification of plants with improved emergence and growth at temperatures ranging from 7 ºC to 22 ºC. Homozygous lines screened for improved

low temperature germination ability could possess greater seedling vigour than those that germinate poorer at low temperatures. King et al. (1986) went on to postulate that since fast germination at low temperatures appears heritable, selection to improve this trait should be possible. Other research into even colder germination temperatures has shown that germination typically does not occur at temperatures lower than 2 ºC (Nykiforuk and Johnson-Flanagan, 1994 and 1997). However, no studies have reported the effect of cold temperature on germination of winter *B. napus* as compared to spring varieties, or the progeny of a cross made between winter and spring germplasm.

3. Materials and Methods

3.1. Original Plant Material and Doubled-haploid Population

All objectives of this research were explored in the context of a single DH population consisting of 115 lines, in addition to the two parental lines (AG-Outback and Caracas). The DH lines of the original population were pre-screened in the greenhouse for the vernalization requirement. Those lines that required vernalization to enter the reproductive phase of growth were removed from the population, resulting in the 115 lines in the experimental group. Due to the segregation for vernalization that occurred within this DH population, it was thought that the population would also segregate for sub-zero temperature tolerance. AG-Outback is a spring *B. napus* variety with Australian origins and adaptability, while Caracas is a winter *B. napus* variety with European origins and a quantitative vernalization requirement. Being a spring type cultivar, AG-Outback grows and matures properly in Saskatchewan, despite its Australian adaptability. In contrast, without vernalization Caracas will remain in the vegetative rosette stage of growth for long periods of time in Saskatchewan prior to bolting. Caracas will eventually flower at approximately the same time as many spring growth-habit lines are finishing flowering in Saskatchewan. Early maturing, open-pollinated check varieties were included in all experiments to control for the effect of the winter background on the agronomic characteristics of the DH lines in this population.

3.2. Field Agronomic Evaluation

3.2.1. Agronomic Evaluation Trial Design and Methodology

In 2012, early spring field trials were designed to capture frost tolerance under spring growing conditions. Trials were planted in the third week of April, and were carried forward due to lack of a killing frost, in order to gather agronomic data through the growing season. In 2013 a late thaw occurred and wet soils resulted in later seeding dates and again, no freezing temperatures (Table 3.1).

Table 3.1. Seeding dates for the agronomic field experiments – 2012 and 2013.

	2012			2013		
	Moon Lake	Rosthern	Watrous	Moon Lake	Rosthern	Watrous
Seeding Date 1	April 24	April 25	April 25	May 8	May 15	May 13
Seeding Date 2	May 12	May 15	May 15	May 21	May 22	May 21

Conventional (non-herbicide tolerant) field practices were used in all trials. All *B. napus* seed was treated with Helix XTra (Syngenta Canada Inc., 20.70% thiamethoxam, 1.25% difenoconazole, 0.39% metalaxyl-M and S-isomer, and 0.13% fludioxonil), a combination fungicide/insecticide seed treatment for early season protection from pests, such as, flea beetles and fungal species, such as, *Pythium* spp*., Fusarium* spp., and *Rhizoctonia* spp. Edge granular herbicide (Dow AgroSciences Canada Inc., 5% ethalfluralin) was applied uniformly pre-seeding as per recommended conventional practices at the rate of 22 kg/ha to control weeds such as green foxtail, kochia, lamb's quarters and wild buckwheat. Granular fertilizer was spread uniformly and incorporated the previous fall at a rate of 90 lb/ac N, 25 lb/ac P and 30 lb/ac S. As a result, no macronutrient deficiencies occurred throughout the trial areas. Additionally, the land used for these trials was harrowed on a 45 º angle to the direction of seeding prior to seeding, to provide a

uniform seed bed. At the appropriate crop staging, conventional *B. napus* herbicide products were applied to minimize weed pressure throughout the trials. Three products were applied in a tank mix as per conventional practices:

1. Centurion® (Bayer CropScience Inc., 240g/L clethodim, a Group 1 herbicide controlling grasses) – 0.125 L/ha

2. Lontrel® (Dow AgroSciences Canada Inc., 300g/L clopyralid, a Group 1 herbicide controlling broadleafs) – 560 mL/ha

3. Muster® (DuPont Canada, Dry flowable Ethametsulfuron-methyl 75%, a Group 2 herbicide controlling broadleafs) – 30 g/ha

If, after application of these products, weeds re-appeared later in the season, they were controlled by manual hoeing between rows and/or tillage of pathways.

3.2.2. Data Collection Strategy

The data collected consisted of common agronomic data points taken throughout the growing season (Table 3.2). Phenological data such as flowering and maturity were taken on multiple occasions to capture the full variation of this population. Traits such as days to flowering and days to maturity were calculated using collected data to facilitate comparison of lines across seeding dates, years and locations. Days to flowering and maturity were computed traits that were derived by subtracting the date of emergence from the date of flower or maturity.

Table 3.2. Summary of agronomic data collected for agronomic experiments.

Collected Data	Calculated Data
Date of seeding	Number of days from emergence to flower
Date of first emergence	Flowering period
Plant stand (1-5 scale, 5 = complete stand)	Number of days from emergence to maturity
Plant vigour (1-5 scale, 5 = strong vigour)	
Date of first flower (50% of row flowering)	
Date of end flower (50% of row complete)	
Date of maturity (50% seed coat colour change, mid-way up the main raceme)	
Plant height (1-5 scale, 5 = tallest entry)	
Lodging (1-5 scale, 5 = standing straight)	
Agronomy (1-5 scale, 5 = excellent)	

3.2.3. Data Analysis

Normalcy of the data was determined by histogram. Further statistical analysis was conducted using descriptive statistics and by ANOVA, fitting a general linear model to the data within the software package R (R Core Team, 2015) using entry, repetition, year and location as primary sources of variation and the interaction terms location*year, location*repetition, entry*location, entry*year and entry*location*year as secondary interaction terms at an alpha (probability of type 1 error) level of 0.01. Further analysis was carried out using the LSD.test function of the R package ‘agricolae’ (de Mendiburu, 2015) whereby the least significant difference between lines was calculated and compared by t-test to generate a chart showing which lines are significantly different using letter codes. The LSD test was carried out using the Bonferroni correction to minimize the probability of type I error occurring with the large number of comparisons being carried out.

3.3. Field Sub-Zero Temperature Tolerance

A second, separate set of trials was planted late in the year, in approximately the third week of August through the first week of September, to capture the potential for a frost event occurring in the fall at the optimal growth stage of 3-6 leaves. In 2012, an unforeseen seed shortage caused the population size to be reduced to 70 DH lines plus three sub-zero temperature tolerant flax lines as sub-zero temperature tolerance checks and the two parents, for a total of 75 lines in the fall trials, plus two local varieties as checks (VT Barrier, SP Banner). The following RCBD trial design was utilized in both 2012 and 2013:

a) Paired rows for all lines
b) Two locations (Moon Lake and Rosthern)
c) Three seeding dates at each location (Table 3.3)
d) Three repetitions per seeding date

As a result of this trial design, 18 effective repetitions were carried out for each DH line that was available for use (nine repetitions per location). In this way, the probability would be reasonably maximized that a damaging frost event would occur at the optimal growth stage of 3-6 true leaves. The first seeding dates occurred on August 24 and 28 in 2012 and August 23 and 26 in 2013, with subsequent seeding dates occurring 7-10 days after the previous seeding date. Data were collected approximately one week following the frosts, to allow for better phenotypic expression. Snowfall late in the season both years prevented note-taking on subsequent major frosts.

Table 3.3. Seeding dates for the fall sub-zero temperature tolerance field experiments in 2012 and 2013.

	2012		2013	
	Moon Lake	Rosthern	Moon Lake	Rosthern
Seeding Date 1	August 24	August 28	August 23	August 26
Seeding Date 2	September 4	September 7	August 30	September 3
Seeding Date 3	September 14	September 17	September 6	September 9

In 2013, the population was expanded to the full group of DHs due to increased availability of seed. The data collection strategies used in 2012 and 2013 are summarized in Table 3.4. Conventional field practices were used in each trial according to the protocol described in section 3.2. The fall sub-zero temperature tolerance field evaluations were not sprayed with herbicides in crop since they would not achieve physiological maturity.

Table 3.4. Data collected during sub-zero temperature tolerance evaluations in fall 2012 and 2013.

	Data Collected Prior to Frost Events	Data Collected After Frost Events
2012	Date of seeding	Plant growth stage (cots or true leaf stage) - evaluated once, day after frost
	Date of first emergence	Plant vigour (1-5 scale, 5 = strong vigour) - evaluated once, day after frost
	Plant stand (1-5 scale, 5 = dense stand)	Chlorosis (1-5 scale, 5 = no chlorosis) - evaluated once, day after frost
	Plant vigour (1-5 scale, 5 = strong vigour)	Necrosis (1-5 scale, 5 = no necrosis) - evaluated once, day after frost
		Re-growth (0-1 scale, 1 = re-growth) - evaluated twice, 5 and 10 days after frost
2013	Date of seeding	Plant growth stage (cots or true leaf stage) - evaluated once, day after frost
	Plant vigour (1-5 scale, 5 = strong vigour)	Plant vigour (1-5 scale, 5 = strong vigour) - evaluated once, day after frost

3.4. Low Temperature Germination Experimental Design

The low temperature germination experiment was carried out using a standard germination test protocol outlined by Nykiforuk and Johnson-Flanagan (1994 and 1997), with minor modifications. In 2012, a preliminary low temperature germination test was carried out in an effort to understand whether significant effects would be found. In a refrigeration chamber and laboratory refrigerator, a randomized complete block design experiment was carried out in which three repetitions of the germination experiment for each entry were randomly placed

within the chamber. All DH seeds selected for use appeared to be good quality, viable seeds that were not cracked or shriveled, and all were sourced from Chile 2011-2012 nursery rows. Because of seed quantities, in 2012 the repetitions of AG-Outback and Caracas were smaller at 50 seeds per rep for AG-Outback and 5 seeds per rep for Caracas.

The seeds for this experiment in 2012 were counted out using a vacuum seed counter and placed in grids on pre-wetted germination blotting paper placed in plastic trays. The seeds were placed at one of three temperatures: 1 ºC in a refrigeration chamber, 4 ºC in a refrigerator, or at 22 ºC in the lab to control for expected germination at normal temperatures. There were two repetitions of all the lines chosen for each treatment. The germination percentages for the two repetitions were measured at 7 days for the 22 ºC treatment and at 14 days for the 1 ºC and 4 ºC treatments, and averaged to find the mean germination percentage for each line at each temperature.

All seeds used in the 2013 low temperature germination trials appeared to be good quality, viable seeds that were not cracked or shriveled, and all were sourced from tented seed increase sources such as bagged greenhouse increases or selfed plants covered using mesh under a tent in the field. The seeds for this experiment were counted out using a vacuum seed counter and placed in grids on pre-wetted germination blotting paper circles placed in 150mm petri dishes. Replications were placed in different temperature treatments that were orthogonally-spaced for ease of data analysis. Temperature treatments included 4 ºC and 12 ºC within refrigeration chambers, and one treatment was at room temperature of 20 ºC to control for germination at an optimum temperature. There were two repetitions of all the lines chosen at each temperature treatment.

3.4.1. Criteria for Selection of Lines

The entries for this experiment were chosen based on the phenotype shown in the 2012 agronomic field evaluations (Table 3.5). The primary phenotype used to determine the entries for the low temperature germination test in 2012 was physiological maturity. Two lines that demonstrated an early to mature, spring-like phenotype and the spring-type parent were chosen to represent that end of the maturity spectrum. Two lines that were consistently near the median maturity date were chosen as intermediate phenotypes that did not display either extreme phenotype. Two additional lines were also chosen which demonstrated a late to mature, winter-like phenotype along with the winter-type parent.

Table 3.5. Entries selected for low temperature germination testing in 2012.

Spring-like Phenotype	Intermediate Phenotype	Winter-like Phenotype
AG-Outback (100)*	NBC11-03855 (200)	Caracas (10)
NBC11-05144 (200)	NBC11-05135 (200)	NBC11-04408 (200)
NBC11-04434 (200)		NBC11-04420 (200)

*Number in brackets is the total seeds tested for each line across two repetitions at each temperature.

In 2013, the procedure for the low temperature germination tests was expanded. Twelve lines were tested, rather than eight, and these lines were selected for testing based on their maturity characteristics as determined through the agronomic assessments carried out in the field in 2012 and 2013. Spring-like, intermediate, and winter-like maturity types were chosen (Table 3.6).

Table 3.6. Entries selected for low temperature germination testing in 2013.

Spring-like Phenotype	Intermediate Phenotype	Winter-like Phenotype
AG-Outback (100)*	NBC11-03855 (100)	Caracas (100)
NBC11-05144 (100)	NBC11-02400 (100)	NBC11-04406 (100)
NBC11-04434 (100)		NBC11-04444 (100)
NBC11-04384 (100)		NBC11-03900 (100)
NBC11-05142 (100)		
VT Barrier (100)		

*Number in brackets is the total seeds tested for each line across two repetitions at each temperature.

The method used for collecting germination data was to count the physical seeds that germinated and compare that to the total number of seeds assayed. Germination counts were performed for each repetition at 7 days after imbibition and 14 days after imbibition for all temperature treatments. The germination percentages were then averaged to find the mean germination percentage for each line at each temperature.

3.4.2. Data Analysis

Following data collection, the raw replicated data were entered into Agrobase Generation II. An ANOVA was completed by fitting a GLM (general linear model) to the data using block (repetition) and entry as fixed effects at an alpha (probability of type 1 error) level of 0.01.

4. Results

4.1. Field Agronomic Evaluations

4.1.1. Days to Emergence

When considering the early first seeding dates of 2012, 85.0% of rows emerged between the 15th and 20th day after seeding (Figure 4.1). The remaining 15.0% of rows emerged between the 20th and 31st day after seeding. One line in particular, NBC11-02751 was consistently among the latest to emerge in each of the three repetitions of the first seeding at the Moon Lake site in 2012. The second seeding dates of 2012 occurred at a more usual seeding time for *B. napus*. Within this group, 32.6% emerged on the 8th, 9th, 10th or 11th day after seeding. The majority of the remainder of rows (65.5%) emerged between the 12th and 15th day after seeding. There was a similar pattern between the first and second seeding dates in 2012.

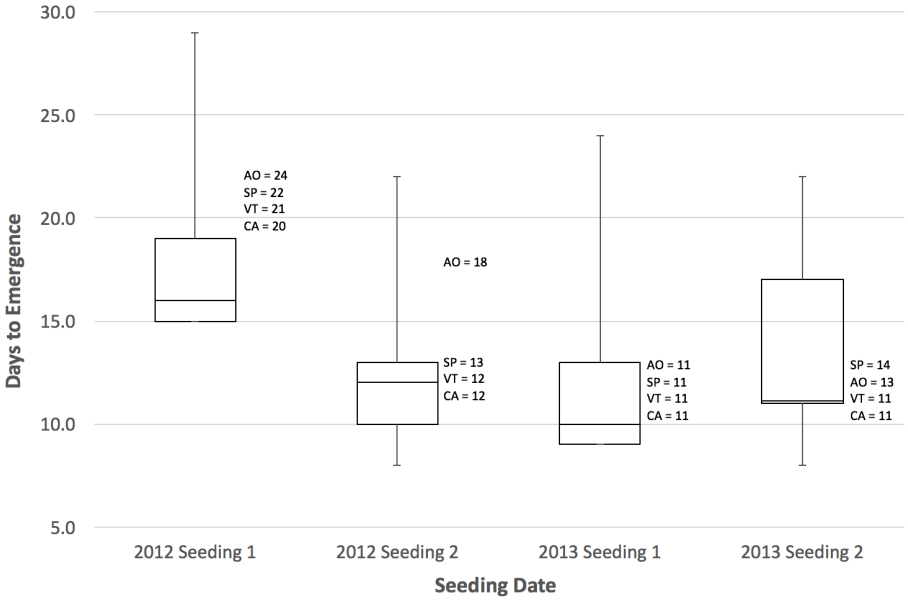

Figure 4.1. Distribution of mean number of days from seeding to emergence for a population of 112 *Brassica napus* DH lines, their two parental lines, and two check varieties, for two seeding dates in spring 2012 and spring 2013. The check varieties' and parental lines' means are indicated by the following abbreviations: VT = VT Barrier, SP = SP Banner, CA = Caracas, AO = AG-Outback.

The first seeding dates of 2013 were roughly equivalent to the second seeding dates of 2012. Within 9-10 days of seeding, 66.9% of rows had emerged, with the majority of the remaining rows (32.1%) emerging on the 13th day after seeding. This timeframe is consistent with the second seeding dates of 2012 (Figure 4.1). The second seeding dates of 2013, being seeded later than any of the other repetitions, emerged the quickest with 16.5% emergence on the 8th day after seeding. On the 11th day after seeding, 66.9% of rows had emerged in the second seeding of 2013. However, although this was the earliest group to emerge in large numbers, a significant number of rows (25.9%) did not emerge until between the 17th and 21st day after seeding (Figure 4.1).

When analysis of both years of data was completed, a number of entries were identified that emerged significantly earlier than some of the checks (Appendix 1, Table A1). AG-Outback was significantly later to emerge than eight of the DH lines tested. Caracas and VT Barrier were not significantly earlier or later to emerge than any of the other tested entries. One DH line, NBC11-02751 was found to be significantly later to emerge than ten of the other DH lines tested.

4.1.2. Early Plant Vigour

The early plant vigour rating was a description of how vigourously a plant emerged from the soil and cycled through the early growth stages prior to bolting. Lines with greater leaf area and a larger diameter rosette stage of growth are rated higher than lines with smaller leaf area and weak overall appearance. The ratings for early plant vigour across all three locations in 2012 and 2013 are summarized below in Figure 4.2.

Little deviation between seeding dates was observed for early plant vigour in 2012. In 2013, a similar trend occurred. The check varieties and parental lines rated consistently close to

four, with their overall means over the two years ranging from 3.4 to 4.1. Overall, the early plant vigour ratings did not deviate greatly between seeding dates within years, or even across years since the same overall trend was noted between seeding dates, between seeding dates across years and between years (Figure 4.2).

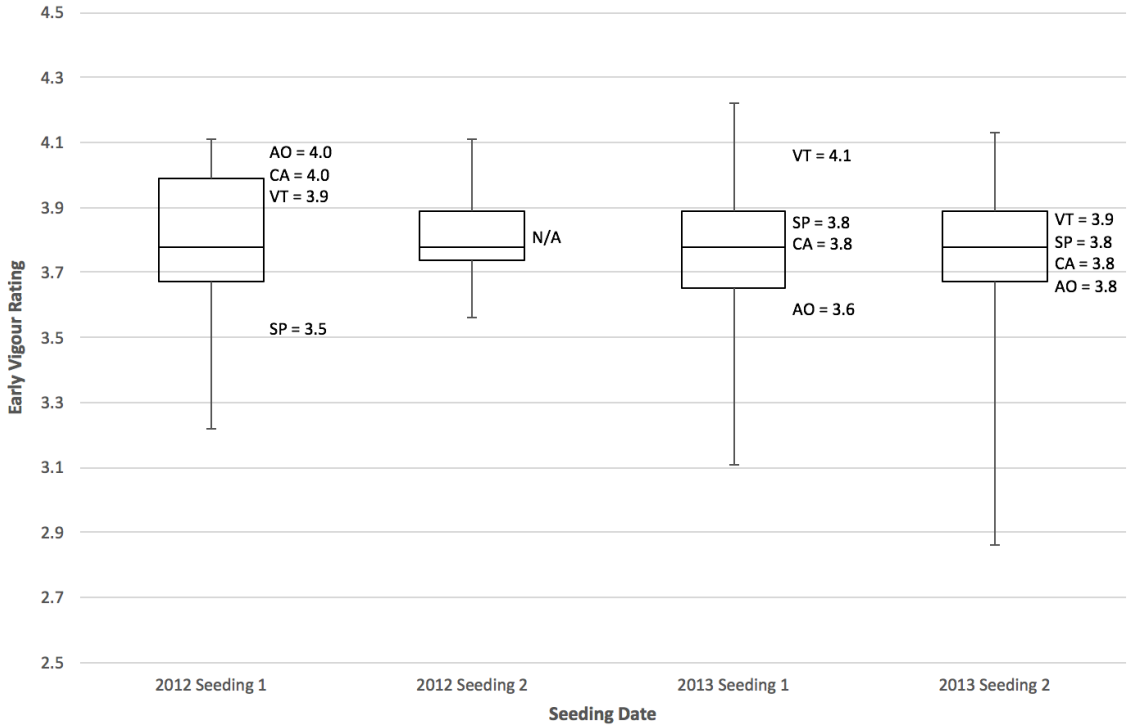

Figure 4.2. Distribution of mean early vigour ratings (1-5 scale, 1=poor vigour and 5=excellent vigour) for a population of 112 *Brassica napus* DH lines, their two parental lines, and two check varieties, for two seeding dates in spring 2012 and spring 2013. The check varieties' and parental lines' means are indicated by the following abbreviations: VT = VT Barrier, SP = SP Banner, CA = Caracas, AO = AG-Outback, N/A = no data.

Multi-year data analysis of the early plant vigour ratings revealed a number of significant differences among the entries. AG-Outback and NBC11-02757 were significantly less vigourous than 52 DH lines within this population (Appendix 1, Table A2). One DH line (NBC11-04464) was significantly more vigourous than thirteen DH lines and both the winter and spring parental lines for this population. Both VT Barrier and SP Banner were found to be not significantly

earlier or later than any of the lines tested in this experiment.

4.1.3. Days to Flowering

Days to flowering data for both years are summarized in Figure 4.3. In 2012 the median days to flower for the first seeding date was up to one week longer than for the second seeding date. Rows planted in the first seeding date began flowering on the 30th day after emergence (Figure 4.3). The last row began flowering on the 64th day after emergence. The median date of first flower occurred 44 days after emergence. The first row began flowering 3 days sooner in the second seeding date than the first seeding date, on the 27th day after seeding. On the 49th day after emergence, all rows had begun to flower. In the second seeding dates of 2012, the median date of first flower occurred 38 days after emergence. The overall distribution for days to flowering for the second seeding dates is narrower than that of the first seeding date because many lines flowered later after emergence in the first seeding date. In 2012, the check varieties VT Barrier and SP Banner were among the first to flower.

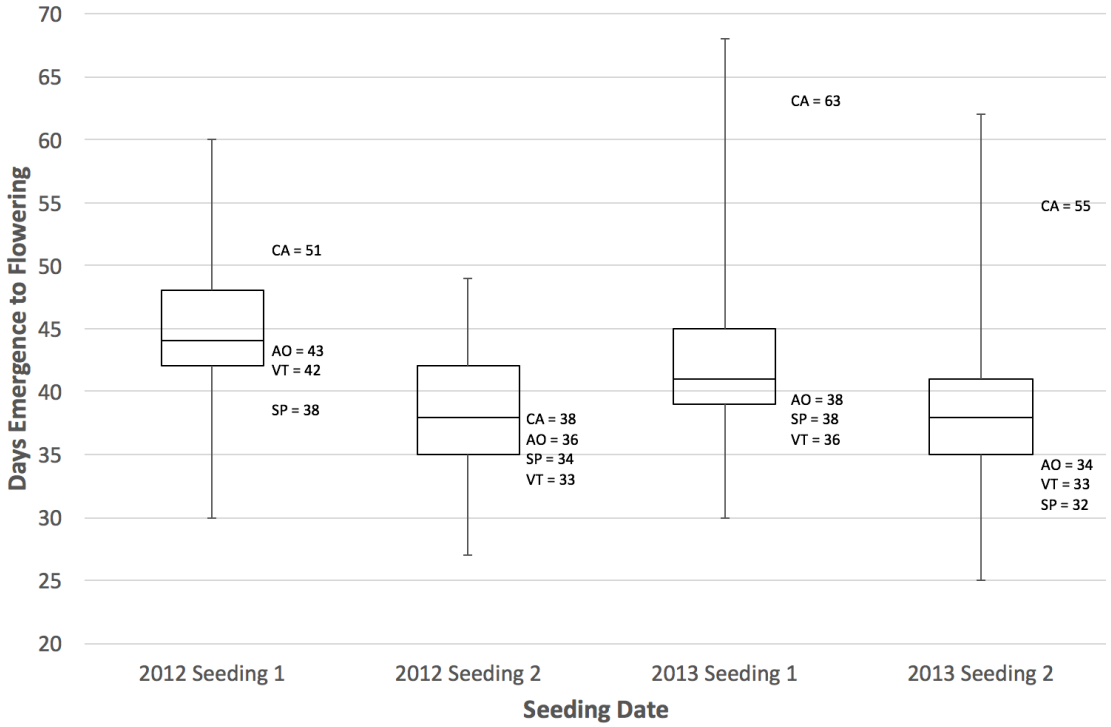

Figure 4.3. Distribution of the mean number of days from emergence to first flower for a population of 112 *Brassica napus* DH lines, their two parental lines, and two check varieties, for two seeding dates in spring 2012 and spring 2013. The check varieties' and parental lines' means are indicated by the following abbreviations: VT = VT Barrier, SP = SP Banner, CA = Caracas, AO = AG-Outback.

In 2013, a similar pattern was observed whereby the median date of flower of the first seeding date group was later after emergence than the second seeding date group. However, the median dates of flower between seeding dates were more similar in 2013 than in 2012. This may be due to the first seeding being completed later in the spring than in 2012. The first seeding group in 2013 started to flower on the 30th day after emergence, while the second seeding group began to flower on the 25th day after emergence (Figure 4.3). The median date of flower for the first seeding date was 41 days after emergence, while for the second seeding date it was 38 days after emergence. On the 68th and 62nd days after emergence, the final rows flowered in the first and second seeding dates, respectfully. These data, along with Figure 4.3 illustrates the delay in flowering that occurred when earlier seeding dates were used. The majority of rows in the second seeding group flowered more quickly than those in the first seeding group. In 2013, the check

varieties VT Barrier and SP Banner and the spring parental line AG-Outback flowered early when compared to the rest of the population. Caracas, the winter parent, was often amongst the latest flowering lines.

When the data from both years are considered together (Figure 4.3) the earlier seeding dates of the first seeding group in 2012 are apparent. The median flowering date of the first seeding of 2012 was the latest of the four seeding dates, and the median flowering date of the second seeding group was earlier than the first seeding group in both years. Caracas was found to be significantly later in days to flower than all other lines within this population (Appendix 1, Table A3). Also, one DH line, NBC11-02386 was found to be significantly later from all DH lines except eight. Both VT Barrier and SP Banner were found to be not significantly earlier from any of the other early maturing lines in this population. However, seven DH lines were found to be significantly earlier to flower and seventeen DH lines were found to be significantly later to flower than the spring parental line, AG-Outback.

4.1.4. Flowering Period

In 2012, the first seeding group showed a narrower distribution than the second seeding group for flowering period, with 89.2% of lines flowering for between 22 and 34 days (Figure 4.4). In contrast, in the second seeding group 89.3% of lines flowered for between 22 and 39 days, a period of time that is five days longer than the first flowering group. Both VT Barrier and SP Banner were found to flower for a relatively short duration compared to the majority of the DH lines in this population, with flowering periods less than 25 days in both the first and second seeding groups. AG-Outback was found to flower longer than the two check varieties, with mean

flowering periods of 37 days for the first seeding group and 30 days for the second seeding group.

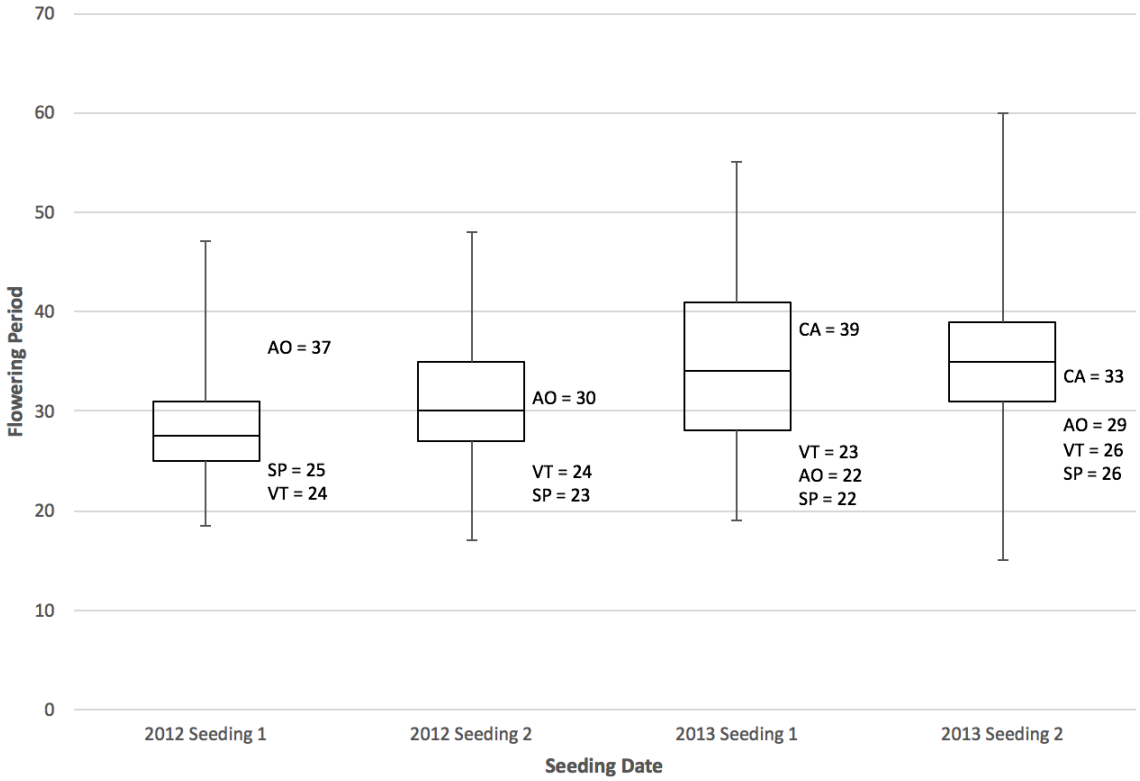

Figure 4.4. Distribution of mean flowering periods for a population of 112 *Brassica napus* DH lines, their two parental lines, and two check varieties, for two seeding dates in spring 2012 and spring 2013. The check varieties' and parental lines' means are indicated by the following abbreviations: VT = VT Barrier, SP = SP Banner, CA = Caracas, AO = AG-Outback.

In 2013, the ranges of flowering period scores for both seeding dates were similar, like in 2012, with 99.4% of all rows flowering for between 19 and 52 days across both seeding dates. While the range of days to flower scores was similar, the distributions within the two seeding groups were different. In the second seeding group, 83.2% of lines flowered for between 27 and 43 days (Figure 4.4). However, in the first seeding group, 83.9% of lines flowered for between 22 and 45 days, a period of time that is 7 days longer than the second seeding group. Similar to 2012, the check varieties flowered for shorter durations than most of the DH lines. VT Barrier, SP Banner and AG-Outback all flowered for less than 24 days in the first seeding group of 2013

and for less than 29 days in the second seeding group of 2013. Caracas, while taking the longest of any line in this population to begin flowering, was not found to have the longest flowering period, with means of 38.7 days of flowering for the first seeding date of 2013 and 32.5 days of flowering for the second seeding date of 2013.

Across years, the two check varieties, VT Barrier and SP Banner, were not significantly longer or shorter in flowering period than the parental line AG-Outback (Appendix 1, Table A4). The flowering periods of VT Barrier and SP Banner were not significantly shorter than the shortest flowering period line NBC11-02385. The flowering period for AG-Outback was significantly shorter than 87 other DH lines in this population. The winter parent, Caracas showed a significantly shorter flowering period than the five longest flowering DH lines, and significantly longer flowering period than the fifteen shortest flowering DH lines as well as VT Barrier, SP Banner and AG-Outback. The DH line with the longest mean flowering period (NBC11-04444) flowered significantly longer than 107 of the DH lines within this population, the parental lines, and the checks. Similarly, the DH line with the second longest mean flowering period (NBC11-04474) flowered significantly longer than 101 of the DH lines, both parental lines, and the checks.

4.1.5. Days to Maturity

In general, the first seeding group of 2012 took longer to mature than the second seeding group, with a median of 102 days. The second seeding group in 2012 had a median of 95 days to maturity (Figure 4.5). The distribution of maturity data for the second seeding group in 2012 was broader and earlier. On the 106th day after emergence, 75% of rows in the first seeding date of 2012 had matured. In contrast, by the 101st day after emergence 75% of rows for the second

seeding date of 2012 had matured. The last row of the second seeding date matured on the 108th day after emergence. In effect, in 2012 the first seeding date and second seeding date matured at approximately the same time, though the first seeding group was planted up to three weeks earlier than the second seeding group, resulting in a longer overall season for the first seeding group.

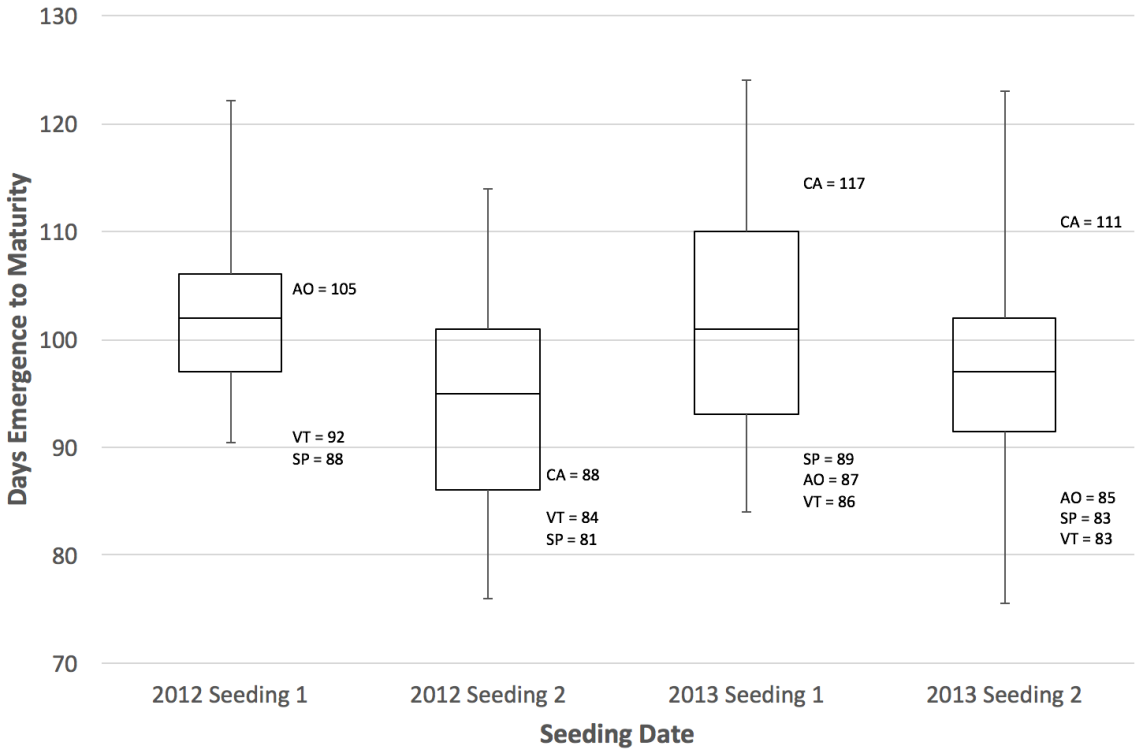

Figure 4.5. Distribution of the mean number of days from emergence to physiological maturity for a population of 112 *Brassica napus* DH lines, their two parental lines, and two check varieties, for two seeding dates in spring 2012 and spring 2013. The check varieties' and parental lines' means are indicated by the following abbreviations: VT = VT Barrier, SP = SP Banner, CA = Caracas, AO = AG-Outback.

When comparing the median days to maturity, the 2013 medians were close to the 2012 medians, however the distributions of the data points were different in 2012 and 2013. For example, the median days to maturity for the first seeding date groups in 2012 and 2013 were 102 and 101 days, respectively. Similarly, the median days to maturity dates for the second

seeding date groups in 2012 and 2013 were 95 and 97 days, respectively. In the first seeding date of 2013 data showed a broader distribution around the mean than the first seeding date of 2012. The second seeding date of 2013 showed a narrower range, but many of the lines matured during a period from 92 to 102 days after emergence, with comparatively fewer lines maturing before or after that period (Figure 4.5). In the first seeding group of 2013, 50% of rows had matured on the 101st day after emergence. In the second seeding group of 2013, 75% of rows had matured on the 102nd day after emergence. On the 122nd day after seeding, all of the rows in the first seeding group had matured, while all the rows in the second seeding group matured by the 125th day. The second seeding group took less time to mature overall than the earlier seeding group.

Further, when comparing the 2012 data to the 2013 data, it is clear that the first seeding groups in both 2012 and 2013 took longer to mature from emergence than the second seeding dates in either year (Figure 4.5). Overall, the data are fairly similar between years for days to maturity, and the differences in range of the data between years could be explained by the extreme earliness of the 2012 seeding dates.

There are a number of significant differences among lines in this population (Appendix 1, Table A5). Caracas was found to be the line within this group that took the longest to mature with a mean of 122 days across both years. This mean was significantly longer than 70 of the DH lines within the population as well as the parental line AG-Outback and two check varieties VT Barrier and SP Banner (Appendix 1, Table A5). Additionally, the second latest line to mature in this population, NBC11-02386, was significantly later than 65 of the DH lines, AG-Outback and the two checks. AG-Outback was found to be significantly earlier to mature than 89 of the DH lines and Caracas, but was not found to be significantly earlier to mature than the earliest DHs or VT Barrier or SP Banner. The days to maturity means of the two checks, VT Barrier and SP

Banner, were found to be not significantly earlier than the mean for the earliest maturing line in the experiment, NBC11-02760. However, VT Barrier was found to be significantly earlier than 97 of the DH lines tested and Caracas, and SP Banner was found to be significantly earlier than 96 of the DH lines tested and Caracas.

4.1.6. Plant Height

Plant height was rated for every row in both 2012 and 2013 on a five-point rating scale from one (extremely short) to five (extremely tall). Of the mean ratings for row height, 0.03% of entries were rated a one, 3.5% of entries were rated a two, 37.0% of entries were rated a three, 46.9% of entries were rated a four and 12.5% of entries were rated a five. The grand mean was 3.6. Caracas had a mean height rating of 5.0, AG-Outback had a mean of 3.0, VT Barrier had a mean of 2.9 and SP Banner had a mean of 2.9. The tallest DH line in the population, NBC11-02386 with a mean height rating of 4.8, was significantly taller than 97 of the DH lines, AG-Outback and the checks. Caracas was found to be significantly taller than 113 out of 115 DH lines in this population as well as AG-Outback and the checks VT Barrier and SP Banner. AG-Outback and VT Barrier were found to be not significantly shorter from the mean of the shortest line in the experiment, SP Banner. The median height ratings for the first and second seeding dates in either 2012 or 2013 were not significantly taller or shorter than each other (Figure 4.6).

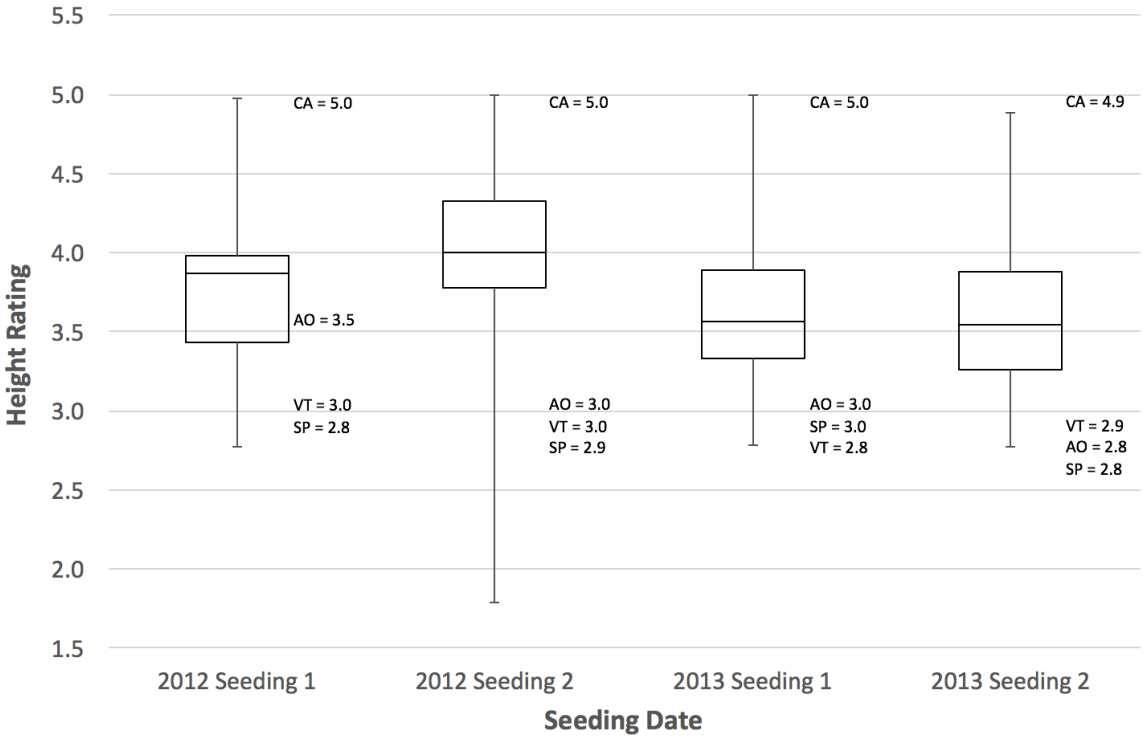

Figure 4.6. Distribution of mean height ratings (1-5 scale, 1=short and 5=tall) for a population of 112 *Brassica napus* DH lines, their two parental lines, and two check varieties, for two seeding dates in spring 2012 and spring 2013. The check varieties' and parental lines' means are indicated by the following abbreviations: VT = VT Barrier, SP = SP Banner, CA = Caracas, AO = AG-Outback.

4.2. Frost Events, Plant Vigour and Phenotypic Observations

Two major frosts occurred at Moon Lake in fall 2012, from which plant vigour data were recorded (Figure 4.7). The first severe frost occurred on October 5 and was -9 ºC. The second frost occurred on October 13 and was -5 ºC. There were other, more moderate frosts before these ones, however no noticeable frost damage occurred as a result of them. After the first severe frost, a near normal distribution of plant vigour scores was observed. As the fall progressed and the second severe frost was recorded, the distribution shifted towards lower plant vigour scores (Figure 4.7). After the first frost, 48.4% of entries scored a 3 (moderate vigour) and 43.4% of entries scored a 4 (strong vigour). After the second frost, 60.0% of entries scored a 3 and only 3.1% of entries scored a 4, while 34.0% of entries scored a 2 (weak vigour). After the first frost,

the grand mean of all ratings was 3.5 with a standard deviation of 0.7. After the second frost, the grand mean of all ratings was 2.6 with a standard deviation of 0.6. The shift from moderate - strong vigour after the first frost to weak - moderate vigour after the second frost underscores the way that *B. napus* seedlings respond to major frost events once acclimated.

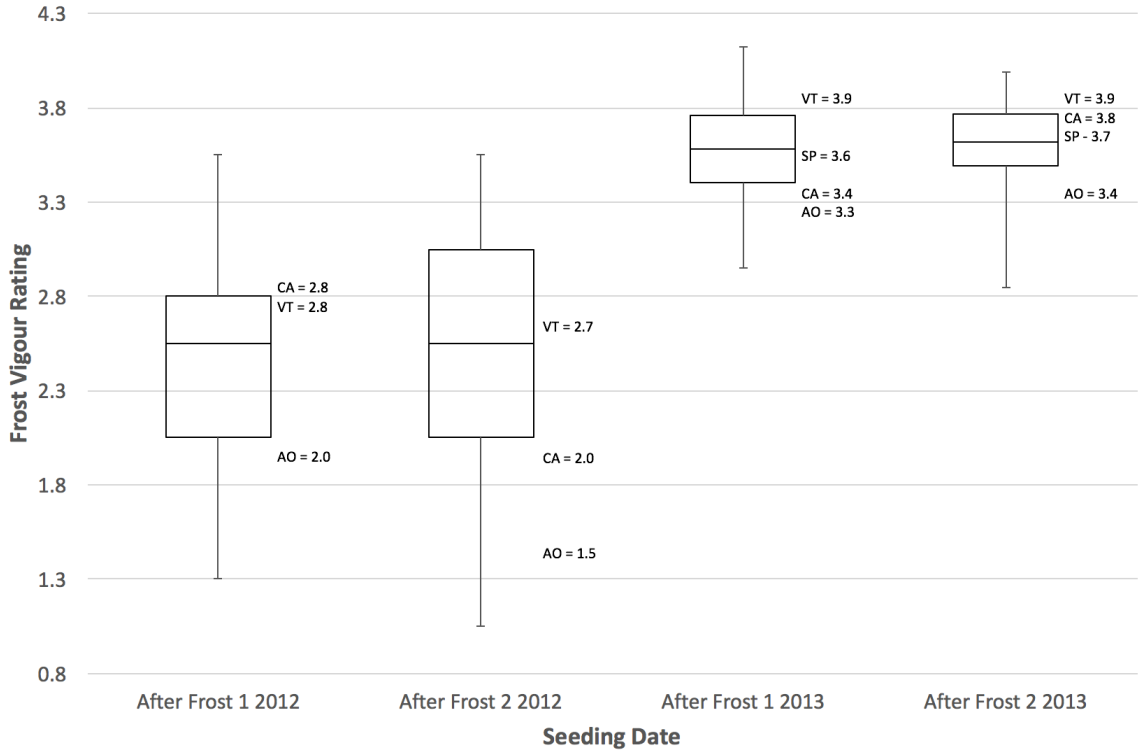

Figure 4.7. Distribution of mean vigour after frost ratings (1-5 scale, 1=poor vigour and 5=excellent vigour) for a population of 112 *Brassica napus* DH lines, their two parental lines, and two check varieties, for two seeding dates in fall 2012 and fall 2013. The check varieties' and parental lines' means are indicated by the following abbreviations: VT = VT Barrier, SP = SP Banner, CA = Caracas, AO = AG-Outback.

In 2013, the fall cold tolerance trial was repeated. At Moon Lake, the first major frost of -3.2 ºC occurred in the early morning hours of October 4, 2013. At Rosthern, the first major frost of -4.5 ºC also occurred at the same time. Notes on these frosts were recorded on October 7, 2013 at Moon Lake, and October 9, 2013 at Rosthern. Minimal phenotypic damage was observed at this time, potentially due to the relative mildness of the frosts combined with cold

acclimation that had occurred prior to the frost event. At Moon Lake, sub-zero temperatures did not occur again until October 12, 2013. From October 12, 2013 until October 17, 2013, the daily low temperatures were colder than -4.5 ºC each night, with the lowest temperature being -6.7 ºC on October 13, 2013. At Rosthern, a similar pattern occured. On October 12, 2013 the temperature fell to -4.8 ºC just before midnight, and for three subsequent nights after that, the lows reached -6.0 ºC, -6.9 ºC and -5.9 ºC, respectively. This period of cold low temperatures did not produce substantial damage at either Moon Lake or Rosthern. At Moon Lake, data was recorded on October 17, 2013 and at Rosthern data was collected on October 23, 2013. Snowfall after October 23, 2013 prevented note-taking on subsequent major frosts.

The data recorded for plant vigour in 2013 did not shift from higher values to lower values as additional frosts occurred, as was observed in 2012. Instead, more lines were rated higher than previous. Prior to the first frost, the vigour scores closely followed a normal distribution, with 17.5% of entries scoring a two, 51.1% of entries scoring a three, and 25.8% of entries scoring a four for vigour. After the first frost, 40.1% of entries scored a three and 56.5% of entries scored a four. After the second frost, 39.5% of entries scored a three and 59.1% of entries scored a four. Only 1.4% of entries scored a two after the second frost. Throughout the multiple frosts observed during this experiment, the mean vigour for the two parental checks (Caracas and AG-Outback) stayed between 3.0 and 4.0, with no damage observed to either parent. Phenotypically, no DH lines appeared to be heavily damaged by the latter prolonged frost event, even one week after the four night event, on October 23, 2013. Due to the cold temperatures, plant growth appeared subjectively to have slowed, however no plants were killed by the frost and heavy frost damage was not observed in the field. After prolonged acclimation, repeated frosts of -6.0 ºC were not cold enough to induce plant death. There was some

phenotypic variability observed in the frost damage by late October, however no plants died as a result of frost damage prior to a snowfall that ended the experiment.

4.3. Low Temperature Germination Experiments

4.3.1. Low Temperature Germination – 2012

The germination percentages of each of the five DH lines and two parents that were tested in 2012 are illustrated in Table 4.1. At 1 ºC, only three lines out of eight germinated at all and even after fourteen days the germination percentages observed were marginal, at best. Of these three lines, NBC11-05144 had the highest germination percentage at 2.8 %, significantly better than all lines with the exception of NBC11-04408. NBC11-04408 had a germination percentage of 1.6 %, not significantly higher than NBC11-03855 at 1.1 %. The standard error for these data at 1 ºC was 0.47 and the LSD was 1.4 %.

Six out of eight lines tested germinated at 4 ºC but germination percentages were still low for all the lines tested, with the exception of NBC11-04420. This line showed better germination percentages at 4 ºC than any of the other lines - 52 % over two repetitions, however, it did not germinate at all at 1 ºC. The other five lines had less than 10 % germination. Caracas and NBC11-05135 did not germinate at all at 4 ºC. The standard error for these data at 4 ºC was 16.75 and the LSD was 50.2 %, however, so the only significant differences found between lines at 4 ºC was between NBC11-04420 and each AG-Outback, NBC11-03855, NBC11-05135 and Caracas. All other comparisons were non-significant.

The 22 ºC control treatment at room temperature provided a baseline germination percentage to establish the quality of the seed for each tested line. All germination percentages at 22 ºC were acceptable and above 90 %, with the exceptions of Caracas and AG-Outback. There

was a seed shortage for the lines AG-Outback and Caracas at the time of the germination test in 2012. Only 2 % of AG-Outback seeds germinated across two repetitions of 50 seeds each, while Caracas had 80 % germination across two repetitions of 5 seeds each. The highest germination percentage of the lines tested at 22 ºC was 97.8 % for NBC11-05135. This line did not germinate at all at 1 ºC or 4 ºC and had been selected for the low temperature germination experiment in 2012 because of its intermediate phenotype. The standard error for these data at 22 ºC was 1.99 and the LSD was 6.0 %. All six DH lines tested had significantly higher germination rates than both AG-Outback and Caracas (Table 4.1). Additionally, NBC11-05135 germinated significantly better than NBC11-03855, NBC11-04434, Caracas and AG-Outback.

Table 4.1. Mean germination percentages for all genotypes tested in 2012 and 2013 at all temperature treatments.

	2012			2013		
Name	1 ºC (LSD=1.4)	4 ºC (LSD=50.2)	22 ºC (LSD=6.0)	4 ºC (LSD=18.0)	12 ºC (LSD=12.0)	20 ºC (LSD=8.1)
Ag-Outback	0	1.1	2	97	94	99
Caracas	0	0	80	56	81	91
VT Barrier	N/A	N/A	N/A	88	99	98
NBC11-04434	0	7	91.3	91	90	75
NBC11-05144	2.8	7.7	93.1	94	93	99
NBC11-04384	N/A	N/A	N/A	81	93	89
NBC11-05142	N/A	N/A	N/A	68	70	90
NBC11-02400	N/A	N/A	N/A	80	89	88
NBC11-03855	1.1	0.5	91.6	100	98	100
NBC11-03900	N/A	N/A	N/A	84	88	96
NBC11-04406	N/A	N/A	N/A	95	97	99
NBC11-04444	N/A	N/A	N/A	85	82	84
NBC11-05135	0	0	97.8	N/A	N/A	N/A
NBC11-04408	1.6	4.1	95.9	N/A	N/A	N/A
NBC11-04420	0	52	95.9	N/A	N/A	N/A

4.3.2. Low Temperature Germination – 2013

In 2013, the low temperature germination test was repeated with minor modifications to generate orthogonal data using more DH lines. The germination percentages throughout the experiment were consistently higher at the 4 ºC temperature than in 2012. At the coldest temperature tested, 4 ºC, ten out of the twelve lines tested scored above 80 % germination after 14 days (Table 4.1). The three lines showing the highest germination percentages were NBC11-03855 at 100 %, AG-Outback at 97 % and NBC11-04406 at 95 % germination. The three lines showing the lowest germination percentages were Caracas at 56 %, NBC11-05142 at 68 % and NBC11-02400 at 80 %. The standard error for these data at 4 ºC was 6.61 and the LSD was 18.0 %. At this LSD level, all the tested entries except NBC11-05142 showed a significantly better germination percentage than Caracas. Additionally, NBC11-03855 germinated significantly better than NBC11-04384 and NBC11-02400 (Table 4.1). NBC11-05142 germinated significantly poorer than AG-Outback, VT Barrier, NBC11-04434, NBC11-05144, NBC11-03855 and NBC11-04406.

The 12 ºC data showed a general trend towards higher germination percentages than the 4 ºC data. The grand mean of the 4 ºC germination percentages was 84.9 % while the grand mean of the 12 ºC germination percentages was 89.5 %. At 12 ºC, eleven out of twelve lines tested scored above 80 % germination after 14 days (Table 4.1). The lowest three germination percentages were NBC11-05142 at 70 % germination, Caracas at 81 % germination and NBC11-04444 at 82 % germination. The highest three germination percentages were VT Barrier at 99 % germination, NBC11-03855 at 98 % germination and NBC11-04406 at 97 % germination. The standard error for these data at 12 ºC was 4.41 and the LSD was 12.0%. At 12 ºC, AG-Outback germinated significantly better than both Caracas and NBC11-05142 (Table 4.1). The

germination percentage of four DH lines (NBC11-05144, NBC11-04384, NBC11-03855 and NBC11-04406) was significantly better than Caracas. Many lines tested showed similar germination percentages at 12 ºC than they did at 20 ºC.

At 20 ºC, the grand mean of all germination percentages was 92.3 % and the individual means ranged from 75% to 100 %. At room temperature, eleven out of twelve lines showed germination percentages higher than 80 % at seven days after imbibition (Table 4.1). Five lines had germination percentages greater than or equal to 98 %: AG-Outback 99 %, VT Barrier 98 %, NBC11-05144 99 %, NBC11-03855 100 %, NBC11-04406 99 %. The only line with a germination percentage lower than 80 % was NBC11-04434 with a germination score of 75 %, which made it significantly worse than all other lines within this experiment. The standard error for these data at 20 ºC was 2.97 and the LSD was 8.1%. AG-Outback germinated significantly better than NBC11-04434, NBC11-04384, NBC11-05142, NBC11-02400 and NBC11-04444 (Table 4.1). Caracas germinated significantly better than NBC11-04434 and significantly worse than NBC11-03855. There were many significant differences in germination percentage among the DH lines at 20 ºC.

Many lines showed non-significant differences in germination percentage among the three temperature treatments completed in this experiment, while some lines showed significant differences among temperatures (Table 4.1). Caracas significantly increased in germination percentage at each step from 4 ºC to 12 ºC to 20 ºC. Importantly, between repetitions, no significant differences were found in 2013, while many significant differences were found among entries.

5. Discussion

5.1. Experimental Conditions

Abiotic stress traits and traits that are strongly influenced by abiotic stresses are particularly difficult for breeders to evaluate and improve due to the precise environmental requirements needed for those traits to express. In a controlled environment, well-designed experiments may be conducted and performance of germplasm may be observed and recorded. However, to screen hundreds or perhaps thousands of lines in a controlled-environment setting is likely to be limited by resources (Reynolds et al., 2001). Even if excellent results were achieved in an indoor setting such as a growth chamber, it is questionable as to whether these results would even be reproducible in a field setting, due to lack of control over the acclimation process typically experienced in the field (Reynolds et al., 2001). Since ultimately there were 115 lines and field evaluation of germplasm has been found to produce more relevant and reliable data (Fowler et al., 1981, 1993), field trials were the method chosen for evaluation of the DH lines. Field evaluation of abiotic stress traits presents its own set of challenges, however; not the least the environment itself. An evaluation of cold tolerance in canola, or any field crop species, requires a strict set of environmental conditions to occur at precisely the right time to properly discern susceptible and tolerant lines. Pertaining to winter survival in winter wheat, this set of conditions have been described as a “test winter” or “differential winter” (Levitt, 1972), but the same principle applies to sub-zero temperature tolerance screening in *B. napus*. Experimental design can only control for some of a number of environmental variables. The required environmental conditions, in this case an appropriate level of cold pre sub-zero exposure

followed by sub-zero temperatures that are low enough to damage susceptible lines but not so low as to kill everything, must still occur to induce the desired stress response.

In 2012 and 2013 the optimal environmental conditions to ideally evaluate sub-zero temperature tolerance in *B. napus* did not occur. In spring 2012, a sub-zero temperature tolerance trial was planted in the third week of April, approximately 3-4 weeks earlier than normal for *B. napus*. However, the temperature did not drop below
0 ºC after seeding and, as such, no sub-zero temperature tolerance data could be collected. Further, because the temperature hovered between 3 ºC and 10 ºC after seeding, emergence was slow. The outcome of the spring 2012 sub-zero temperature testing highlights a complicating factor surrounding testing for sub-zero temperature tolerance in the spring. When seeding early in the spring, cold temperatures in the ambient air and in the soil inhibit rapid germination and emergence, even if all other conditions are optimal. Once the plants do emerge, it may be mid-May, at which time frosts become less regular and even less predictable. Due to environmental challenges, it is difficult to assess sub-zero temperature tolerance in the spring using frosts as the evaluation metric. The number of days from seeding to emergence may be a better indication of tolerance to cold ambient temperatures and soil. As a result of this experience, trials for tolerance to sub-zero temperatures were switched to fall seedings.

In fall 2012, a frost of approximately -9 ºC occurred in the early morning hours of October 5, but by that date the plants had well-acclimated to the cooling overnight temperatures. That evening, temperatures were below 0 ºC for approximately seven hours, with temperatures below -5 ºC for approximately two hours. However, in fall 2013, sub-zero temperatures colder than -6 ºC did not occur until after snowfall covered the plants. In fall 2012 and fall 2013, major plant death was not observed. *B. napus* seems to possess a relatively strong, inherent tolerance to

cold at certain growth stages when properly acclimated, as seen in fall 2012 and fall 2013 when only minor phenotypic variations were observed. This is a similar result to those obtained by Kirkland and Johnson (2000) and Johnson et al. (1995), who observed survival of spring temperatures from -6 ºC to -8ºC in fall-seeded, acclimated seedlings. In order to observe statistically significant variation, a relatively warm and consistent fall would be required. At some point, a rapid drop in temperature would have been required, to at least a low temperature of -8 ºC. A hypothesis is that the combination of a low potential for acclimation and a damaging frost could result in greater phenotypic clarity and more definitive results. The other potential outcome of such an event may be a total loss of all plants involved. Such uncertainty further underscores the volatility of attempting to evaluate sub-zero temperature tolerance in a field setting. If such an event occurred, where a damaging frost followed little chance for acclimation, that event would be quite rare and perhaps a poor reflection of the most common real-world circumstances.

Testing for low temperature germination is less fraught with complications due to the ability to control the experimental environment. When the same temperature conditions and the same seed lots are used for each repetition, repeatable data will be obtained. In Petri dishes, the germination paper and seeds were not allowed to dry at all, and a consistent level of moisture was provided throughout the entire test. Additionally, the temperature treatments were regulated closely, monitored daily, and adjusted accordingly to maintain the desired temperature throughout the experiment. Because of these factors, high levels of germination were recorded even at low temperatures. Significant differences in germination were observed, however, and some lines performed significantly better than others. While this is a positive outcome, there is still a level of uncertainty due to the variation in seed quality among the lines. For example, in

the 20 ºC germination tests in 2013, mean germination percentages ranging from 75 % to 100 % were recorded (Figure 4.9). Even though uniform, round seeds of the highest quality were chosen for inclusion in this experiment, the germination rate of *B. napus* seed is highly dependent upon the initial seed quality and environmental conditions during storage (Elias and Copeland, 1994). Different seed lots of the same line will show differential germination based on the environment of the year during which they were created (Gusta et al., 2004), and also how they were stored. As such, it is difficult for germination test scores to be repeatable when different seed lots had to be used. The results of the germination test for this study were, none the less, informative.

The agronomic component of this study yielded informative data related to the phenotypic variability of a DH population created from a winter by spring cross. Significant variation was observed from emergence and early establishment through to physiological maturity and harvest. Significant differences were observed in almost all traits, among the DH lines in the population, between the parents of the population, and between the DH lines and the parents. While not all easily accessible or well-adapted to Western Canadian growing conditions, this research agrees with others such as Rahman and Kebede (2012) regarding the amount of genetic diversity that is available within *B. napus* should winter germplasm be utilized. Working to incorporate and evaluate diverse germplasm into spring *B. napus* will be necessary to derive continuous improvements in not only the traits discussed, but all traits of interest.

5.2. Field Agronomic Evaluation Trials

The days to emergence was highly dependent on the environment and was related to the earliness of seeding. The earliest seeding group in 2012 took the longest to emerge from the soil, while the late groups emerged sooner than the early groups in both 2012 and 2013. After 2012, it

was determined that sub-zero temperature tolerance would not be assessed in the spring. Therefore, the seeding dates for the 2013 seeding groups were moved back into a more appropriate timeframe for *B. napus* seeding. Also, a wetter spring in 2013 than in 2012 prevented seeding any earlier. The second seeding dates in 2012 are roughly equivalent to the first seeding dates in 2013. Emergence occurred within the narrowest window of time for the first seeding group of 2013, with comparatively fewer rows taking longer than 13 days to emerge than any other seeding date.

From an agronomic standpoint, of the four seeding dates, the first seeding date of 2013 was the most optimal in terms of temperature and moisture, because the vast majority of rows emerged quickly and uniformly. The second seeding group of 2013 did not emerge nearly as uniformly as the first, with many rows taking over 17 days to emerge, likely due to less soil moisture being present than at the first seeding date. A timeframe of 15-30 days between seeding and emergence is considerably longer than normal for *B. napus,* which will typically emerge in 7-10 days under adequate field moisture and temperature conditions (Gusta et al., 2004 and Kirkland and Johnson, 2000).

While VT Barrier and AG-Outback had among the highest germination percentages at 12 ºC testing, they both had later mean days to emergence in the field than a number of DHs in the population. AG-Outback had a higher mean days to emergence score and was also significantly later to emerge than eight DHs in the population, even though it had the second highest mean germination percentage at 4 ºC in the 2013 low temperature germination test. While AG-Outback germinates well at low temperatures, it is possible that it does not grow vigourously enough to emerge from the soil quickly. Poor early season vigour within the Western Canadian

environment is a characteristic of AG-Outback that has been observed repeatedly by others (Dr. A.D.W. Grombacher, pers. comm.).

The days to emergence data generated in this experiment indicates that there is an optimal seeding window for *B. napus* in the first half of May, because the mean days to emergence in that period of time was the lowest of the seeding times tested (Figure 4.1). This agrees with some previous research and contradicts others. Kirkland and Johnson (2000) argued that there are significant yield advantages to fall-seeded or April-seeded *B. napus* in that emergence earlier in the season benefits the whole growth cycle. The major reason for this is it allows the crop's flowering period to avoid the typically hot and dry period in July that can inhibit proper seed formation (Kirkland and Johnson, 2000). This is similar to the conclusions made by Degenhardt and Kondra (1981) which indicate that seeding late in May results in significant yield disadvantages. Gusta et al. (2004) indicate that seed produced by plants sown the previous fall or in April is larger in diameter, has higher germination percentages and also greater seedling vigour. The benefits to seeding earlier in the season described above were not seen in the first seeding date of 2012 in this study. That may be attributable to different environmental conditions, and because seeding dates in April were only used in 2012, more data would be necessary to make a definitive conclusion.

The early plant vigour rating distributions were similar in both years. Given consistent seed quality, there is no reason to expect that early vigour scores for an individual genotype or population would change based on year unless the environmental conditions between years varied drastically. Significant frosts or severe temperature fluctuations can cause emergence or early vigour reductions in canola (Kirkland and Johnson, 2000). While one seeding date was early in the case of this study, the plants did not actually emerge until just before the second

seeding group did. Therefore, in 2012 and 2013, the plants experienced similar growing conditions above ground once they emerged. As such, early vigour scores did not deviate greatly between seeding groups or between years. The overall early vigour of this population, including the checks and parents, was fairly strong, with grand means from each seeding date of approximately 3.8. VT Barrier and SP Banner are considered to be lines with strong early vigour (Dr. A.D.W. Grombacher, pers. comm.), and there were no DH lines in this population that were significantly more vigourous from them, though there were a number of lines that showed significantly higher vigour than AG-Outback specifically.

From a breeding perspective, it would be beneficial if sub-zero temperature tolerance, low temperature germination, and strong early vigour could be assembled together. This appears to be possible, as the lines NBC11-04458 and NBC11-04419 possessed among the highest early vigour scores (significantly better than AG-Outback, Appendix I, Table A2) and were also among the best lines for sub-zero temperature tolerance (significantly better than VT Barrier and AG-Outback, Appendix I, Tables A7, A8). That these quantitative traits of interest can be gathered together after a single winter by spring cross is encouraging, especially when the cross progeny perform significantly better than one of its parents and a strong check cultivar. However, assessment of later-season agronomic traits of interest is also necessary to determine the breeding value of these identified lines.

Often, the earlier a line is able to begin the flowering process, the earlier it will be able to mature at the end of the season. However, as noted in section 2.4.1, extreme earliness often leads to lower yields due to less time spent in the reproductive phase of growth producing seed. This idea is contrasted by Kirkland and Johnson (2000) who observed that earlier seeded, or even fall-seeded, *B. napus* reached flowering earlier, spent a longer period of time in the reproductive

stage of growth, and reached maturity sooner. In their experiments, Kirkland and Johnson (2000) were able to observe emergence earlier in April than occurred in this research. Of the four seeding dates in the current experiment, the first seeding date of 2012 stands out as being later to flower than the other seeding dates, though the first seeding dates in both years took longer to reach flowering from emergence (Figure 4.3). While the other three seeding groups were all substantially in flower by the 40th day after emergence, the first seeding group of 2012 was just beginning to flower. While the difference in days to flower between the first and second seeding dates is larger in 2012 than 2013, the shape of the data and distributions are similar between the two years. In both years, the second seeding dates flowered sooner after seeding than the first seeding dates. This is the same result that occurred when Degenhardt and Kondra reported a similar experiment in 1981. Their results indicated flowering up to seven days earlier for canola seeded on May 31, versus May 3 (Degenhardt and Kondra, 1981). A probable explanation for the longer time to flower measured in the first seeding dates is that the colder ambient air and soil temperatures during the early seeding dates led to slower growth rates prior to flowering. Another explanation is that *B. napus* possesses an inherent ability to compensate for late seeding, by shortening each growth stage up to maturity (Degenhardt and Kondra, 1981). However, this compensation was not adequate to completely make up a period of 14 days between seeding dates. (Degenhardt and Kondra, 1981). Kirkland and Johnson (2000) observed different results, in which earlier seeded *B. napus* was able to flower earlier than later seeded repetitions of the same trial.

AG-Outback was later to flower in the first seeding group of 2012, perhaps due to poor seed quality, though it was usually slightly later to flower than VT Barrier and SP Banner (Figure 4.3). Caracas was significantly later to flower than all of the DHs in this population,

which would be expected given its winter pedigree and quantitative vernalization requirement. In contrast, VT Barrier and SP Banner were not significantly earlier to flower than the earliest lines in this population, though they are considered to be early cultivars (Dr. A.D.W. Grombacher, pers. comm.). A consistent cohort of DH lines appears to possess significantly superior sub-zero temperature tolerance, quicker days to emergence and quicker days to flower than at least one of the parental lines. This group includes lines the following lines noted above: NBC11-04419, NBC11-02385, NBC11-04464, NBC11-02760, and NBC11-04458 (Appendix I, Table A3). This data demonstrates that it is possible to obtain lines from a winter by spring cross that will flower early enough to be usable in a commercial breeding program, and still have increased cold tolerance and strong early vigour. These positive agronomic traits enable the material to be crossed with elite inbreds possessing other traits, or used directly to make experimental hybrids.

It was expected that a greater number of flowers could lead to a higher number of pods, such that flowering period would be an indirect indicator of yield. However, it has been previously determined that flowering period is not directly related to seed yield (Degenhardt and Kondra, 1981) and that seeding date does not significantly affect flowering period (Kirkland and Johnson, 2000). Instead, days to first flower is considered a more important indicator of yield (Degenhardt and Kondra, 1981). The distribution of flowering period measurements in the current experiment was narrower in 2012 than in 2013, perhaps due in part to the earlier seeding dates. The first seeding date of 2012 in particular showed a distinctly narrower distribution of ratings than any of the three other seeding dates. A possible hypothesis is that this is due to how early this group was planted combined with the environmental conditions of 2012. The second seeding group of 2012 also showed a narrower distribution of ratings than either seeding group in 2013. As such, the flowering period in 2012 was shorter than the flowering period in 2013 by

approximately one week (Figure 4.4). As would be expected, the difference in mean flowering period between years was greater than between seeding dates within a given year. Kirkland and Johnson (2000) also indicate that flowering period was not affected by seeding date.

Interestingly, Caracas takes significantly longer than all other lines to begin flowering, but does not flower for the longest duration amongst this population. Five DH lines in this population had significantly longer flowering periods than Caracas, and fifteen lines, in addition to the checks and AG-Outback, flowered for a period of time significantly shorter than Caracas. The lines previously noted in this discussion for positive agronomic traits, NBC11-02385, NBC11-04419, NBC11-04458, NBC11-03888 and NBC11-02760, are also part of this group that did not flower significantly longer than the check varieties and AG-Outback (Appendix I, Table A4). This group of lines contains increased tolerance to sub-zero temperatures, as well as a growing number of positive agronomic traits related to early maturity. Some DH lines in this population flowered for significantly longer than the vast majority of other DHs as well as the checks. Line NBC11-04444 flowered significantly longer than 107 DH lines, SP Banner, VT Barrier, AG-Outback and Caracas (Appendix I, Table A4). However, it was not significantly later to being flowering than VT Barrier or SP Banner (Appendix I, Table A3). The above results considered together imply it is possible to combine the traits for flowering earliness and flowering period to potentially maximize the amount of flowers that can be produced in a growing season, or also combine increased sub-zero temperature tolerance and early maturity with a winter genetic background. This indicates that the positive agronomic characteristics of the spring parent can be combined together with the genetic diversity of the winter parent efficiently. For a breeding program, this provides reassurance that further crosses between winter

and spring material may be carried out without worrying that no viable material may emerge from them.

While the first seeding group of 2012 took approximately seven days longer to mature from seeding than the second seeding group, both groups began to mature at approximately the same time of the year. The second seeding group was not delayed by the cool soil and air temperatures endured by the first seeding group early in the season. In 2013, more lines matured in a shorter period of time in the second seeding group, whereas the days to maturity scores for the first seeding group were more widely distributed (Figure 4.5). Previous studies have found that later seeded *B. napus* matures as many as 5 days earlier than the same cultivars seeded up to 30 days earlier (Degenhardt and Kondra, 1981). Those results concur with the current experiment, in which the later seeding dates matured significantly earlier than the earlier seeding dates. In contrast, Kirkland and Johnson (2000) observed earlier seeding dates maturing significantly earlier than later seeding dates.

Overall, this population can safely be described as skewing towards later maturity, and many of the lines are too late to be commercially viable. This phenomenon in a winter by spring population has been observed previously (Rahman and Kebede, 2012). Caracas and three DH lines, NBC11-02386, NBC11-04444 and NBC11-04413 had the latest mean days to maturity measurements, all with values greater than 120.0 days (Appendix I, Table A5). There was incomplete data for Caracas in 2012 due to low plant stands. On the other end of the spectrum, AG-Outback, SP Banner, VT Barrier and seven DH lines displayed mean days to maturity measurements of less than 100.0 days (Appendix I, Table A5). Of these seven DH lines that did not have significantly later maturities from the check varieties and AG-Outback, five are familiar lines from earlier in this discussion. Lines NBC11-02760, NBC11-02385, NBC11-04419,

NBC11-04458 and NBC11-04464 were all lines identified to possess increased tolerance to sub-zero temperatures, strong emergence times, early flowering and short flowering period, as well as now early maturity. These lines that take less than 100 days to mature could be commercially usable from an agronomic standpoint in a *B. napus* hybrid breeding program, and also possess increased sub-zero temperature tolerance and the diverse winter genetic background.

Height ratings were taken on this population because winter germplasm is known to have a taller, later, and more robust growth habit than spring germplasm (Rahman and Kebede, 2012). Unsurprisingly, the winter parent Caracas was found to have the tallest growth habit, which was significantly taller than all but the two tallest DH lines by mean height rating, NBC11-02386 and NBC11-04473. AG-Outback, VT Barrier and 41 other DH lines were found to be not significantly taller than the shortest line in the experiment, SP Banner (Appendix I, Table A6). VT Barrier and SP Banner are considered to be average height lines (Dr. A.D.W. Grombacher, pers. comm.), and with mean ratings of 2.86 for SP Banner and 2.92 for VT Barrier the mean ratings support that conclusion. The overall mean height ratings did not differ significantly between seeding dates in either 2012 or 2013 (Figure 4.6). However, Kirkland and Johnson (2000) found that overall mean height was reduced with earlier seeding dates. This was not observed in the current experiment, which may be partly explained by the winter by spring nature of this population. The fact that this is a winter by spring population becomes evident when considering that all the mean height ratings for DH lines were greater than that of SP Banner.

Height and biomass are important to *B. napus* in that a sturdy and robust plant with substantial leaf area is required for capable pod set, standability and high yield (Thurling, 1974). However, taller height and greater biomass do not necessarily equal greater yield and often result

in later maturity due to increased time necessary to accommodate the extra growth. Genotypes possessing short vegetative periods yield higher (Degenhardt and Kondra, 1981). A robust base of rapid vegetative growth to build from, coupled with a longer reproductive period (mid-flower to physiological maturity) are most important to overall yield. Indeed, the tallest DH, NBC11-02386, is also the latest DH line to mature in this population. Phenotypic observations of these taller and more vegetative lines indicated that stem diameters and accumulated biomass (stem, leaves, non-reproductive tissue) were greater in the later to mature lines. In addition to the obvious growing season length limitations, this phenotype would not be conducive to effective harvesting as greater diameter stems and extra biomass would require the equipment to work harder and potentially increase breakdowns. The same familiar DH lines detailed above in each preceding discussion section (NBC11-02760, NBC11-04458, NBC11-04419, NBC11-04464, NBC11-02385 and NBC11-03888) were not significantly taller from the check varieties or AG-Outback (Appendix I, Table 4.6). What is ultimately desirable from a phenotypic standpoint is a robust plant that is able to stand well at average height and produce high quantities of seed in a small, densely architected area. Breeding for this phenotype as well as increased sub-zero temperature tolerance, within the context of a winter by spring population, appears feasible given the results obtained in these experiments.

A primary goal of this research at the outset was to determine whether sub-zero temperature tolerance could be introgressed from the winter germplasm into a spring-like growth habit to increase the sub-zero temperature tolerance and diversity of the existing spring *B. napus* germplasm, without bringing along the undesirable traits of the winter parent such as later maturity and increased biomass accumulation. The first part of this question is whether sub-zero temperature tolerance is even part of the winter heterotic pool that could be contributed by the

winter parent. Information provided in the literature indicates that sub-zero hardiness is a trait of the winter heterotic pool that has been actively selected for in the European region (Rahman and Kebede, 2012). However, since Caracas did not emerge significantly sooner than any other line in this population, and also germinated significantly poorer at 4 ºC than almost all the lines tested, it is difficult to conclude using only the current results that sub-zero temperature tolerance is exhibited more clearly by the winter *B. napus* germplasm, or more specifically, Caracas. The spring parent, AG-Outback, performed significantly better than Caracas in the low temperature germination tests (Figures 4.8, 4.9) and was not significantly earlier or later to emerge than Caracas (Appendix I, Table A1). The second part of the above hypothesis concerns the ability of the descendants of a winter by spring cross to exhibit increased sub-zero temperature tolerance while still displaying the agronomic profile of a spring *B. napus* line, and harbouring some of the genetic diversity provided by the winter parent. This directly relates to the usability of such a line in a commercial breeding program, which should be the underlying and biggest-picture goal behind any field-based plant breeding research. By the results indicated and discussed above, these hypotheses should be accepted. Further, the original hypothesis that the DH lines generated from this winter by spring cross would be significantly different from either of the parents should be accepted. As discussed, many DH lines from this population not only possess, but combine traits that are significantly improved and different from the parental lines.

Ideally, a plant breeder would be looking to find the best attributes of all the aforementioned agronomic traits studied within one breeding line or even better, multiple lines. Because of the inherent genetic complexity of the agronomic traits studied here, it may be unlikely that such an event would occur in a single breeding cycle (one simple cross and one resultant population). However, as demonstrated previously above, in the context of this

population that combination of the best agronomic characteristics did in fact occur. From a breeding perspective, it may be useful to examine some selected lines within this population from this frame of reference to see if any other interesting combinations resulted from this cross.

In this research, as noted above, the most conclusive data with regard to tolerance to sub-zero temperatures is the low temperature germination data and days to emergence data. Upon examining all the agronomic traits above for the highest and lowest means, only one of the DH lines tested in the low temperature germination tests had significantly high or low means for any of the traits. NBC11-04444 showed a below average low temperature germination percentage, was not significantly later to flower than VT Barrier or SP Banner, and possessed a long flowering period and late maturity. This line was originally selected for the low temperature germination tests because of its winter-like phenotype. Similar to Caracas, it did not germinate well at any of the temperatures tested, though it germinated consistently between 80-85% at all three temperature treatments. Despite its earliness to flower, NBC11-04444 was late to mature because of its extended flowering period. This result demonstrates that it can be either a late start to flowering (Caracas) or a long flowering period (NBC11-04444) that influences late maturity. No other lines from the low temperature germination experiment showed significantly high or low means for any other traits. Two lines, however, showed significantly early days to emergence scores and also a number of other significantly high or low means in other categories. Line NBC11-04419 shows increased sub-zero temperature tolerance, is early to emerge, early to flower with a short flowering period, and has early maturity and short height. As noted repeatedly above, line NBC11-02385 also demonstrated good sub-zero temperature tolerance, early emergence, high vigour, early flowering, short flowering period and early maturity. These two lines are excellent agronomic models for selection, further evaluation, and potentially

crossing. These two lines were not tested for low temperature germination. Line NBC11-02766 showed a similar flowering pattern to NBC11-04444 (not significantly later to flower than any lines, but significantly longer flowering period than most) but was not significantly earlier or later to mature than many other lines.

Three other lines stood out as they had combined a number of traits that made them either spring-like or winter-like. The earlier maturing, spring-like phenotypes appear to possess better sub-zero temperature tolerance overall. Line NBC11-04458 had good sub-zero tolerance, good early vigour, was early to flower with a short flowering period, and was early to mature and short. Line NBC11-03872 was early to flower with a short flowering period, early to mature and short. Line NBC11-02386 was late to flower, late to mature and was tall. When seeking the extreme high or low means for these traits, the lines that appear in multiple traits show that the traits segregate for either a spring-like or winter-like phenotype in all cases. There are not, for example, tall and early maturing lines, or early to flower and short duration of flower, but late maturing lines. Physiologically this makes sense, and is also corroborated by existing literature (Rahman and Kebede, 2012). From a breeding perspective, it is important to understand that when making winter by spring crosses, from a pool of approximately 100 DHs it is possible to select out individual lines that are not significantly different agronomically from VT Barrier or SP Banner but also carry within them a significant compliment of winter genetics to increase sub-zero temperature tolerance and boost heterotic potential.

5.3. Field Evaluation of Tolerance to Sub-zero Temperatures

The fall cold tolerance trials provided better environmental conditions but still did not result in any measureable plant death due to frost. Two major frosts occurred in the fall of 2012.

After the first frost, many lines showed vigour scores of three or four out of five. After the second frost, these ratings shifted to scores of two or three and the grand mean of all ratings fell from 3.46 to 2.63 (Figure 4.7). This indicated a decrease in overall vigour after the second frost compared to after the first frost. However, in 2013 a similar shift in vigour scores did not occur. After multiple frosts in October 2013, vigour ratings increased. Perhaps the reason for this discrepancy is that the plant growth stages at which the frosts occurred were different in 2012 and 2013. In 2013, the fall seedings were completed on a more compressed timeline than in 2012. In both years, the seedings started at the same approximate time, but in 2013 the third seeding date was seeded approximately one week earlier than in 2012. Coupled with warmer environmental conditions, this difference led to approximately a 3-4 leaf stage difference between the plants in 2012 and in 2013. This difference in leaf stage, and the potential for greater acclimation in 2013 could explain part of the variation in cold tolerance results across the two years. A difference in acclimation speed and potential between plants of different growth stages was previously noted by Schilling (2004) and Gusta and O'Connor (1987).

The objective of evaluating sub-zero temperature tolerance in this population was to determine whether significantly improved tolerance could be generated by this specific cross. While perfect environmental conditions to evaluate this trait did not occur at either of the locations in either year of the experiment, some useful conclusions can still be made. In both years, SP Barrier showed the best sub-zero temperature tolerance of the parents and check varieties, while AG-Outback performed the worst. No lines from this DH population outperformed SP Banner. However, there were a number of lines that showed significantly better tolerance to sub-zero temperatures than one or both of the parents over both years: NBC11-04419, NBC11-04458, NBC11-04459 showed significantly better sub-zero temperature tolerance

than both VT Barrier and AG-Outback after the first and second frosts, and NBC11-02385, NBC11-02760, NBC11-03844, NBC11-03888 and NBC11-04409 showed significantly better sub-zero temperature tolerance than VT Barrier and AG-Outback after the first frost, and AG-Outback after the second frost (Appendix I, Tables A7, A8). Days to emergence in cold soil is also a good indicator of cold tolerance. From the lines mentioned above, NBC11-02385 and NBC11-04419 were among the earliest lines to emerge from the soil, and were significantly earlier than both SP Banner and AG-Outback (Appendix I, Table A1). One other line (NBC11-04409) from the above group was found to be significantly earlier to emerge than SP Banner (Appendix I, Table A1).

These results, when considered together, indicate that it is indeed possible to derive breeding lines from a winter by spring cross such as this, with significantly improved cold tolerance over the spring parent (AG-Outback). The hypothesis for this section of the research should, therefore, be accepted.

Sub-zero temperature acclimation of *B. napus* plants in both laboratory and field environments has been tested before, particularly by Schilling in 2004 and Rapacz in 2002. *B. napus* plants were acclimated under field or laboratory conditions to temperatures of -17 ºC (spring canola) and -18 ºC (winter canola) by both researchers (Schilling, 2004 and Rapacz, 2002), though Schilling (2004) reported that acclimation under field conditions did not quite reach these temperatures. The 1 ºC difference in acclimation between the spring and winter types was not found to be significant (Schilling, 2004). However, this result could have been a product of the fall environmental conditions that his plants were subjected to. The results of Schilling and Rapacz clearly indicate that when properly acclimated, *B. napus* plants have an ability to survive cold temperatures down to -15 ºC in field conditions. This information may explain why

significant plant death did not occur in my populations, even when subjected to multiple overnight periods approaching -10 ºC. Further, it has been postulated that both spring and winter canola possess the major genes required for sub-zero temperature tolerance, but the two types interpret environmental conditions differently to activate the cold tolerance response and subsequent acclimation (Gusta and Wisniewski, 2013). My results suggest that genotypic differences in sub-zero temperature tolerance can exist between DH lines derived from a winter by spring cross in *B. napus*. Gusta et al. (2001) also reported that genotypic differences in acclimation response exist between winter wheat cultivars. If Gusta and Wisniewski's (2013) hypothesis that spring and winter types interpret the environment differently to govern their acclimation response is correct, my results further show that this environmental interpretation pattern may be heritable and selectable due to the genotypic differences seen in my population.

When considered together, the above information and my results indicate that it is possible to select for and improve sub-zero temperature tolerance and cold acclimation response in *B. napus*. Likewise, it also seems that the winter-type germplasm is a plausible source of genetic diversity to achieve improved tolerance to sub-zero temperatures. A likely next step in the breeding process would be to select the highest performing lines (such as those eight lines indicated above) for sub-zero temperature tolerance from this population and continue to cross or backcross them to early maturing and well-adapted elite germplasm. While there are a few lines in this population that are early maturing and also have good sub-zero temperature tolerance (see section 5.4), larger well-adapted populations could be derived through additional rounds of crossing/backcrossing and selection.

5.4. Low Temperature Germination Testing

In the 2012 low temperature germination experiment, less than 2% of seeds germinated at the 1 ºC temperature treatment (Figure 4.8) indicating that this temperature is too low for reliable germination of *B. napus* seeds. There were, however, some seeds that germinated. These were likely the most robust seeds that were exposed to optimal moisture conditions. The DH line NBC11-05144 performed significantly better than all other entries at the higher temperatures in this experiment. At 4 ºC, all the germination percentages observed were less than 10%, with the exception of NBC11-04420, which had 52% germination. As a result of the wide variation in germination percentage for NBC11-04420 at 4 ºC, the standard deviation and LSD for this genotype were high and no meaningful results could be extracted other than that NBC11-04420 germinated significantly better at 4 ºC than the rest of the entries in one of the two repetitions (Figure 4.8). Even at 4 ºC, germination of *B. napus* seeds is inhibited or perhaps too slow to measure in a standard germination test protocol. This is supported by the results achieved by Kondra et al. (1983) where *B. napus* seeds took extended time periods to germinate at temperatures lower than 5 ºC. Low temperatures combined with darkness, even in the presence of adequate moisture, may induce seed dormancy in *B. napus* (Lopez-Granados and Lutman, 1998), which could partially account for low germination percentages at low temperatures.

The control temperature of 22 ºC showed that the chosen seed lots germinated well under optimal conditions, with the exception of AG-Outback. This line did not germinate well at 22 ºC either, with a mean germination percentage of 2% over 100 seeds. As a result, the 2012 data for AG-Outback at the cold temperature treatments of 1 ºC and 4 ºC is not an indicator of actual performance. The best performing line overall in 2012 was NBC11-05144, which showed the highest germination percentages at 1 ºC and at 4 ºC, and had an acceptable 22 ºC control

germination. NBC11-05144 was also a line that showed early maturity in the 2012 and 2013 agronomy evaluations, with mean days to flower and days to maturity measurements that were not significantly later than the early check varieties VT Barrier and SP Banner. Line NBC11-05144 was not significantly different from the two check varieties or AG-Outback in any other agronomic category. This makes NBC11-05144 a candidate line that possesses spring-like agronomic characteristics such as early maturity, and also above average low temperature germination.

The results of the low temperature germination testing experiment in 2013 were more conclusive than the 2012 results. Some lines showed variation among temperature treatments, and some lines behaved similarly at all three temperatures (Figure 4.6). As would be expected, the highest mean percentage germination across all lines was at 20 ºC, while the lowest mean percentage germination across all lines was at the 4 ºC temperature treatment. Caracas scored the poorest germination at 4 ºC but did substantially better at 12 ºC and even better at 20 ºC. Caracas may be better adapted to germinating in warmer soil due to being a winter cultivar that is typically planted in the fall. Conversely, AG-Outback germinated well at all temperatures and performed significantly better than Caracas at both 4 ºC and 12 ºC. AG-Outback may germinate better in cold temperatures because it is adapted to being planted into colder soils in the spring (Dr. W. Burton, pers. comm.). VT Barrier scored the highest mean germination percentage at 12 ºC as it is a Canadian-bred, early spring cultivar that is known for fast emergence from the soil. While AG-Outback germinates quickly at low temperatures, it is worth noting that its early vigour and days to emergence scores are among the lowest in this population (Appendix I, Tables A1, A2).

Two lines did not show significant differences across any of the temperature treatments. NBC11-03855 had between 95-100% germination across all temperature treatments, while NBC11-04444 had 80-85% germination at all temperature treatments. Line NBC11-04444 may have only germinated up to 85% of seeds due to seed quality. Caracas may have been similarly affected. One line, NBC11-04434 germinated better at the cold temperatures than at 20 ºC. Because NBC11-04434 behaves in an opposite way to Caracas, it could be hypothesized that it does so because it is a spring-adapted line that tolerates colder germination conditions better than a winter-adapted line that would typically be planted into cold soils.

One additional aspect of the low temperature germination testing is that specific lines were chosen for this experiment due to the diversity of their phenotypes in the field. In 2012 three spring, two intermediate and three winter phenotypes were chosen for testing. The two spring-like DH lines chosen, NBC11-05144 and NBC11-04434 performed better than five of the eight lines tested at 4 ºC. NBC11-04420 was a winter-like DH line and it performed significantly better than all other lines at 4 ºC in 2012. The two intermediate lines chosen, NBC11-03855 and NBC11-05135 performed poorly at both 1 ºC and 4 ºC, however NBC11-05135 scored the highest germination percentage in 2012 at the 22 ºC temperature treatment. There were no clear trends in the 2013 data as to whether winter/spring phenotype played any role in germination performance. The worst performing DH line in the experiment was NBC11-05142, a spring-like line. Additionally, an intermediate phenotype line, NBC11-03855 performed the best out of any of the lines tested at 4 ºC and second best at 12 ºC. NBC11-04406, a winter-like line, also performed well at both 4 ºC and 12 ºC. These results indicate that the low temperature germination trait may segregate independently of other agronomic traits of interest.

The hypothesis for the low temperature germination experiment was that DH lines in this population would exist that germinated better under cold conditions than either of the two parents. Eight out of nine lines tested in 2013 germinated significantly better than the winter parent, Caracas. Therefore, the hypothesis for this section of the research should be accepted.

Low temperature germination ability and seedling cold tolerance are traits that are required for rapid emergence in cold soil. Improved cold tolerance leads to greater seedling vigour in cold soil, which helps to push the plant through the soil more rapidly to emerge quicker. Other research suggests that the earlier canola can be planted in the spring, the higher the yield potential may be, due to cooler temperatures and greater moisture availability during the flowering period (Kirkland and Johnson, 2000). If cold tolerance, strong early vigour, and faster emergence times in the early spring were bred for, those varieties could have a head start and potential yield advantage over those that required warmer soils to germinate and emerge. By measuring days to emergence early in the field evaluation regime of an inbred line, low temperature germination and cold tolerance could be selected for and bred for in successive generations. The other agronomic characteristics of those lines could potentially all be favourable as well (see section 5.4), such that direct crosses with minimal agronomic penalties may be made between early to emerge lines.

6. Summary and Conclusions

In recent years, plant breeding efforts in *B. napus* within Western Canada have broadened to include more diverse genetic resources such as winter germplasm. While hurdles exist that must be overcome (Rahman et al., 2011), increasing germplasm diversity is widely regarded as a method by which gains can be made in a number of important traits including hybrid yield, disease resistance and perhaps improved tolerance to abiotic stresses (Rahman and Kebede, 2012). While increased vigour and tolerance to sub-zero temperatures in the early spring would benefit this crop, improved sub-zero temperature tolerance in spring *B. napus* introgressed from winter germplasm is an area in which limited field research has been done to date.

The results of this research indicate that it is possible for the spring-like phenotype and improved low temperature germination, tolerance to sub-zero temperatures and early vigour to be combined together. A number of experimental lines within this population also demonstrated that it is possible for significantly poorer low temperature germination and lower days to emergence to be associated with both the late to mature winter-like phenotype and the early to mature spring-like phenotype. The results further demonstrated that the characteristics of low temperature germination, early vigour, flowering and maturity are not inherited together in any combination and can be manipulated individually. Conceivably, using a larger population size or perhaps a different breeding method and a larger experimental scope it would be possible to select many more individuals that combine all the positive traits of interest noted above. This means that as an approach to broaden the genetic diversity of the spring *B. napus* germplasm, incorporating winter by spring crosses into a commercial breeding program is a viable option.

However, to fully understand the utilization of this material in a commercial breeding program, a number of other traits such as disease resistance and seed oil profile would also have to be evaluated. Only if these traits were also present in addition to all the agronomic characters mentioned above, would it make sense to convert these lines into CMS lines for hybrid evaluation. In cases where a DH line possesses only one or two extraordinary characteristics, additional crossing or backcrossing work could be done to further adapt the material.

Less than ideal field environmental conditions during the experiments of this research led to usable but less than conclusive results in the area of sub-zero temperature tolerance. This is the nature of field work targeting abiotic stress traits, although additional locations and years could help mitigate this issue somewhat. Future work in this area could investigate other winter by spring populations to determine whether similar results are found using other winter parents. Identifying QTL (quantitative trait loci) that influence sub-zero temperature tolerance or low temperature germination could be beneficial to breeding programs wishing to select for these traits in their germplasm. However, this type of genomic work requires extensive and accurate phenotypic analysis in the field, which remains a major challenge for abiotic stress traits year to year. Controlled-environment experiments may yield usable phenotypic data, but whether quantitative trait loci identified and selected through the use of such phenotypic data would translate to similar performance in the field should also be investigated. Finally, since canola seed is sold in Western Canada as F1 hybrid seed, the levels of heterosis obtained using winter by spring breeding lines as hybrid mother and father lines should be evaluated to determine the usability of germplasm of this type commercially.

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8. Appendices

Appendix I: LSD Tables

Table A1. Mean days to emergence for DH lines and checks in 2012 and 2013 field evaluations. Lines with the same significance letter code are not significantly different. The mean, standard deviation, lower confidence level (LCL), upper confidence level (UCL), minimum value (MIN) and maximum value (MAX) are reported.

significance	*NAME*	*MEAN*	*STDEV*	*LCL*	*UCL*	*MIN*	*MAX*
a	SP Banner	**15.26**	5.89	14.22	16.29	9	31
ab	NBC11-02751	**14.91**	5.84	13.91	15.91	8	29
abc	Ag-Outback	**14.88**	5.93	13.70	16.05	8	29
abcd	NBC11-05138	**14.32**	5.04	13.29	15.36	9	29
abcd	NBC11-03826	**14.31**	5.23	13.30	15.33	8	29
abcd	NBC11-04392	**14.09**	5.50	13.09	15.09	8	30
abcd	NBC11-02754	**14.06**	4.61	13.05	15.08	8	26
abcd	NBC11-04403	**13.97**	5.05	12.95	14.99	8	30
abcd	NBC11-03906	**13.94**	4.25	12.90	14.97	9	24
abcd	NBC11-05135	**13.91**	4.82	12.89	14.92	8	29
abcd	CARACAS	**13.88**	5.03	12.86	14.89	8	29
abcd	NBC11-02396	**13.82**	4.55	12.82	14.82	8	29
abcd	NBC11-03897	**13.79**	4.47	12.79	14.79	8	28
abcd	NBC11-05130	**13.79**	4.04	12.79	14.79	8	25
abcd	NBC11-03843	**13.73**	3.45	12.68	14.78	9	19
abcd	NBC11-02398	**13.72**	3.85	12.70	14.74	9	22
abcd	NBC11-02753	**13.72**	4.61	12.70	14.74	8	29
abcd	NBC11-02759	**13.63**	4.54	12.61	14.64	8	29
abcd	NBC11-05143	**13.63**	4.84	12.61	14.64	8	28
abcd	NBC11-03827	**13.59**	4.00	12.58	14.61	9	23
abcd	NBC11-03823	**13.58**	3.72	12.55	14.61	9	21
abcd	NBC11-03876	**13.52**	3.99	12.51	14.52	8	24
abcd	NBC11-04408	**13.52**	4.49	12.51	14.52	8	27
abcd	VT Barrier	**13.52**	4.49	12.51	14.52	8	25
abcd	NBC11-03847	**13.48**	3.47	12.48	14.49	9	19
abcd	NBC11-04483	**13.45**	3.79	12.45	14.46	8	21
abcd	NBC11-05148	**13.45**	4.14	12.45	14.46	8	25
abcd	NBC11-03718	**13.44**	4.49	12.42	14.45	8	30
abcd	NBC11-02391	**13.42**	4.24	12.42	14.43	8	27
abcd	NBC11-04406	**13.42**	4.89	12.42	14.43	8	29
abcd	NBC11-05144	**13.42**	3.56	12.42	14.43	9	19
abcd	NBC11-04420	**13.41**	4.56	12.39	14.42	8	30
abcd	NBC11-04452	**13.36**	3.89	12.36	14.37	8	23
abcd	NBC11-04400	**13.31**	3.87	12.30	14.33	8	24
abcd	NBC11-04454	**13.30**	3.98	12.30	14.30	8	24
abcd	NBC11-02384	**13.28**	4.33	12.26	14.30	8	24
abcd	NBC11-03855	**13.25**	3.91	12.23	14.27	8	21
abcd	NBC11-02400	**13.22**	4.46	12.20	14.24	8	29
abcd	NBC11-04433	**13.22**	3.83	12.20	14.24	8	22
abcd	NBC11-02397	**13.21**	4.14	12.21	14.21	8	23
abcd	NBC11-03842	**13.16**	3.65	12.14	14.17	8	22

abcd	NBC11-04434	**13.12**	3.43	12.12	14.12	8	19
abcd	NBC11-04384	**13.06**	3.45	12.06	14.06	8	19
abcd	NBC11-04484	**13.03**	3.47	12.03	14.03	8	20
abcd	NBC11-02387	**13.00**	3.93	12.01	13.99	8	23
abcd	NBC11-04462	**13.00**	3.84	11.98	14.02	8	22
abcd	NBC11-03879	**12.97**	3.71	11.97	13.97	8	21
abcd	NBC11-05142	**12.81**	3.21	11.80	13.83	8	18
abcd	NBC11-03828	**12.77**	3.57	11.72	13.82	8	20
abcd	NBC11-02399	**12.53**	4.03	11.05	14.02	9	21
abcd	NBC11-04435	**12.40**	3.76	10.91	13.89	9	21
abcd	NBC11-03888	**12.29**	3.34	10.75	13.82	9	19
abcd	NBC11-02388	**12.27**	3.47	10.78	13.75	9	20
abcd	NBC11-03858	**12.27**	3.45	10.78	13.75	9	19
abcd	NBC11-04436	**12.27**	3.45	10.78	13.75	9	19
abcd	NBC11-02401	**12.21**	3.98	10.68	13.75	9	21
abcd	NBC11-03838	**12.21**	3.66	10.68	13.75	9	21
abcd	NBC11-04471	**12.15**	3.34	10.56	13.75	9	19
abcd	NBC11-03833	**12.07**	3.67	10.53	13.61	9	21
abcd	NBC11-04413	**12.07**	3.32	10.53	13.61	9	19
abcd	NBC11-03872	**11.93**	3.29	10.39	13.47	8	19
abcd	NBC11-04476	**11.92**	3.75	10.33	13.52	9	22
abcd	NBC11-03874	**11.86**	2.98	10.32	13.40	9	18
abcd	NBC11-03884	**11.86**	3.16	10.32	13.40	9	19
abcd	NBC11-03822	**11.80**	3.75	10.31	13.29	8	22
abcd	NBC11-03859	**11.80**	2.60	10.31	13.29	9	17
abcd	NBC11-04457	**11.80**	2.65	10.31	13.29	9	17
abcd	NBC11-02386	**11.73**	3.17	10.25	13.22	8	19
abcd	NBC11-02760	**11.73**	3.56	10.25	13.22	8	21
abcd	NBC11-04474	**11.73**	2.74	10.25	13.22	8	17
abcd	NBC11-03856	**11.67**	2.94	10.18	13.15	9	19
abcd	NBC11-02752	**11.64**	2.68	10.10	13.18	9	17
abcd	NBC11-03837	**11.64**	2.92	10.10	13.18	9	20
abcd	NBC11-03890	**11.62**	2.99	10.02	13.21	9	20
abcd	NBC11-02757	**11.60**	3.33	10.11	13.09	8	21
abcd	NBC11-04459	**11.60**	3.44	10.11	13.09	8	20
abcd	NBC11-03845	**11.57**	3.55	10.03	13.11	8	21
abcd	NBC11-03900	**11.57**	3.18	10.03	13.11	8	19
abcd	NBC11-03832	**11.53**	3.25	10.05	13.02	8	21
abcd	NBC11-03844	**11.53**	3.07	10.05	13.02	8	19
abcd	NBC11-03869	**11.53**	3.07	10.05	13.02	8	19
abcd	NBC11-04391	**11.53**	3.07	10.05	13.02	8	19
abcd	NBC11-04418	**11.53**	3.07	10.05	13.02	8	19
abcd	NBC11-04485	**11.53**	3.07	10.05	13.02	8	19
abcd	NBC11-02389	**11.47**	2.90	9.98	12.95	8	18
abcd	NBC11-03835	**11.47**	2.90	9.98	12.95	8	18
abcd	NBC11-04488	**11.47**	2.90	9.98	12.95	8	18
abcd	NBC11-04428	**11.43**	3.16	9.89	12.97	8	19
abcd	NBC11-02766	**11.40**	2.75	9.91	12.89	8	17
abcd	NBC11-03853	**11.40**	2.53	9.91	12.89	8	17
abcd	NBC11-04458	**11.40**	2.75	9.91	12.89	8	17
abcd	NBC11-04479	**11.40**	2.75	9.91	12.89	8	17
abcd	NBC11-03885	**11.36**	2.98	9.82	12.90	8	18
abcd	NBC11-04464	**11.31**	2.68	9.87	12.75	8	17
abcd	NBC11-03903	**11.29**	2.20	9.75	12.82	9	17
abcd	NBC11-04437	**11.29**	2.46	9.75	12.82	8	17
abcd	NBC11-03861	**11.27**	2.52	9.78	12.75	8	18

abcd	NBC11-04430	**11.27**	2.49	9.78	12.75	8	17
abcd	NBC11-04467	**11.20**	3.26	9.71	12.69	8	21
abcd	NBC11-03898	**11.14**	3.35	9.60	12.68	8	21
abcd	NBC11-04463	**11.14**	2.41	9.60	12.68	8	17
abcd	NBC11-04396	**11.07**	2.84	9.53	12.61	8	19
bcd	NBC11-03717	**11.00**	2.27	9.51	12.49	8	17
bcd	NBC11-04473	**11.00**	2.83	9.40	12.60	8	19
bcd	NBC11-04444	**10.87**	2.59	9.38	12.35	8	18
bcd	NBC11-02765	**10.80**	2.40	9.31	12.29	8	17
bcd	NBC11-03864	**10.80**	2.40	9.31	12.29	8	17
bcd	NBC11-03893	**10.80**	2.40	9.31	12.29	8	17
bcd	NBC11-03902	**10.80**	2.40	9.31	12.29	8	17
cd	NBC11-04409	**10.64**	2.13	9.10	12.18	8	15
cd	NBC11-03854	**10.54**	1.66	8.94	12.13	8	13
d	NBC11-04432	**10.47**	1.81	8.98	11.95	8	13
d	NBC11-03905	**10.42**	1.62	8.76	12.08	8	13
d	NBC11-04389	**10.36**	1.74	8.82	11.90	8	13
d	NBC11-04456	**10.36**	1.74	8.82	11.90	8	13
d	NBC11-04487	**10.36**	1.74	8.82	11.90	8	13
d	NBC11-02385	**10.20**	1.78	8.71	11.69	8	13
d	NBC11-02755	**10.20**	1.78	8.71	11.69	8	13
d	NBC11-04419	**10.20**	1.78	8.71	11.69	8	13

Table A2. Mean early vigour rating for selected significantly different lines across both 2012 and 2013 field evaluations. Lines with the same significance letter code are not significantly different. The mean, standard deviation, lower confidence level (LCL), upper confidence level (UCL), minimum value (MIN) and maximum value (MAX) are reported.

significance	*NAME*	*MEAN*	*STDEV*	*LCL*	*UCL*	*MIN*	*MAX*
a	NBC11-04464	**4.11**	0.32	3.90	4.32	4	5
ab	NBC11-04458	**4.06**	0.24	3.85	4.26	4	5
abc	NBC11-02385	**4.00**	0.00	3.79	4.21	4	4
abcd	NBC11-03872	**4.00**	0.50	3.78	4.22	3	5
abcd	NBC11-04391	**4.00**	0.00	3.79	4.21	4	4
abcd	NBC11-04419	**4.00**	0.00	3.79	4.21	4	4
abcd	NBC11-04454	**4.00**	0.28	3.83	4.17	3	5
abcd	NBC11-04479	**4.00**	0.34	3.79	4.21	3	5
abcd	NBC11-02760	**3.94**	0.24	3.74	4.15	3	4
abcd	NBC11-03897	**3.93**	0.27	3.76	4.10	3	4
abcde	NBC11-02401	**3.89**	0.32	3.68	4.10	3	4
abcde	NBC11-02765	**3.89**	0.32	3.68	4.10	3	4
abcde	NBC11-03832	**3.89**	0.32	3.68	4.10	3	4
abcde	NBC11-03844	**3.89**	0.58	3.68	4.10	3	5
abcde	NBC11-03869	**3.89**	0.32	3.68	4.10	3	4
abcde	NBC11-04389	**3.89**	0.47	3.68	4.10	3	5
abcde	NBC11-04452	**3.89**	0.32	3.72	4.06	3	4
abcde	NBC11-05130	**3.89**	0.32	3.72	4.06	3	4
abcdef	NBC11-04456	**3.88**	0.49	3.67	4.10	3	5
abcdef	NBC11-04406	**3.85**	0.36	3.68	4.02	3	4
abcdef	NBC11-02753	**3.84**	0.37	3.66	4.02	3	4
abcdef	NBC11-02755	**3.83**	0.38	3.62	4.04	3	4
abcdef	NBC11-03856	**3.83**	0.38	3.62	4.04	3	4
abcdef	NBC11-03861	**3.83**	0.38	3.62	4.04	3	4
abcdef	NBC11-03884	**3.83**	0.38	3.62	4.04	3	4
abcdef	NBC11-03903	**3.83**	0.38	3.62	4.04	3	4

abcdef	NBC11-04430	**3.83**	0.51	3.62	4.04	2	4
abcdef	NBC11-04444	**3.83**	0.38	3.62	4.04	3	4
abcdef	NBC11-04474	**3.83**	0.38	3.62	4.04	3	4
abcdef	NBC11-03845	**3.82**	0.39	3.61	4.04	3	4
abcdef	NBC11-04396	**3.82**	0.53	3.61	4.04	3	5
abcdef	NBC11-04471	**3.82**	0.39	3.61	4.04	3	4
abcdef	NBC11-02397	**3.81**	0.40	3.64	3.99	3	4
abcdef	NBC11-04434	**3.81**	0.40	3.64	3.99	3	4
abcdef	NBC11-03826	**3.81**	0.40	3.63	3.98	3	4
abcdef	NBC11-02388	**3.78**	0.43	3.57	3.99	3	4
abcdef	NBC11-02399	**3.78**	0.43	3.57	3.99	3	4
abcdef	NBC11-03822	**3.78**	0.43	3.57	3.99	3	4
abcdef	NBC11-03835	**3.78**	0.43	3.57	3.99	3	4
abcdef	NBC11-03855	**3.78**	0.58	3.61	3.95	3	5
abcdef	NBC11-04409	**3.78**	0.43	3.57	3.99	3	4
abcdef	NBC11-04436	**3.78**	0.43	3.57	3.99	3	4
abcdef	NBC11-04457	**3.78**	0.43	3.57	3.99	3	4
abcdef	NBC11-04463	**3.78**	0.43	3.57	3.99	3	4
abcdef	NBC11-05142	**3.78**	0.42	3.61	3.95	3	4
abcdef	NBC11-05144	**3.78**	0.42	3.61	3.95	3	4
abcdef	NBC11-05135	**3.77**	0.76	3.60	3.94	1	5
abcdef	NBC11-02396	**3.74**	0.53	3.57	3.91	2	4
abcdef	NBC11-04403	**3.74**	0.53	3.57	3.91	2	4
abcdef	NBC11-04462	**3.74**	0.45	3.57	3.91	3	4
abcdef	NBC11-05143	**3.74**	0.66	3.57	3.91	1	4
abcdef	NBC11-03843	**3.73**	0.53	3.56	3.90	2	4
abcdefg	NBC11-03717	**3.72**	0.46	3.51	3.93	3	4
abcdefg	NBC11-03833	**3.72**	0.46	3.51	3.93	3	4
abcdefg	NBC11-03874	**3.72**	0.46	3.51	3.93	3	4
abcdefg	NBC11-03885	**3.72**	0.67	3.51	3.93	2	5
abcdefg	NBC11-04459	**3.72**	0.67	3.51	3.93	2	5
abcdefg	NBC11-04473	**3.72**	0.46	3.51	3.93	3	4
abcdefg	NBC11-03828	**3.72**	0.54	3.54	3.90	2	4
abcdefg	NBC11-03838	**3.71**	0.47	3.49	3.92	3	4
abcdefg	NBC11-04476	**3.71**	0.47	3.49	3.92	3	4
abcdefg	NBC11-04487	**3.71**	0.47	3.49	3.92	3	4
abcdefg	NBC11-02400	**3.70**	0.54	3.53	3.87	3	5
abcdefg	NBC11-03879	**3.70**	0.47	3.53	3.87	3	4
abcdefg	NBC11-02759	**3.69**	0.55	3.52	3.87	2	4
abcdefg	NBC11-02386	**3.67**	0.69	3.46	3.88	2	4
abcdefg	NBC11-02398	**3.67**	0.55	3.50	3.84	2	4
abcdefg	NBC11-03718	**3.67**	0.48	3.50	3.84	3	4
abcdefg	NBC11-03837	**3.67**	0.59	3.46	3.88	2	4
abcdefg	NBC11-03842	**3.67**	0.55	3.50	3.84	3	5
abcdefg	NBC11-03847	**3.67**	0.62	3.50	3.84	2	4
abcdefg	NBC11-03853	**3.67**	0.49	3.46	3.88	3	4
abcdefg	NBC11-03854	**3.67**	0.49	3.46	3.88	3	4
abcdefg	NBC11-03859	**3.67**	0.49	3.46	3.88	3	4
abcdefg	NBC11-03893	**3.67**	0.49	3.46	3.88	3	4
abcdefg	NBC11-03900	**3.67**	0.59	3.46	3.88	2	4
abcdefg	NBC11-04384	**3.67**	0.48	3.50	3.84	3	4
abcdefg	NBC11-04418	**3.67**	0.49	3.46	3.88	3	4
abcdefg	NBC11-04484	**3.67**	0.55	3.50	3.84	2	4
abcdefg	NBC11-04488	**3.67**	0.49	3.46	3.88	3	4
abcdefg	NBC11-05148	**3.67**	0.55	3.50	3.84	2	4
abcdefg	VT Barrier	**3.67**	0.55	3.50	3.84	3	5

abcdefg	NBC11-04437	**3.65**	0.61	3.43	3.86	2	4
abcdefg	NBC11-04408	**3.63**	0.63	3.46	3.80	2	4
abcdefg	NBC11-04420	**3.63**	0.63	3.46	3.80	2	4
abcdefg	NBC11-04433	**3.63**	0.63	3.46	3.80	2	4
abcdefg	NBC11-02389	**3.61**	0.70	3.40	3.82	2	4
abcdefg	NBC11-03858	**3.61**	0.61	3.40	3.82	2	4
abcdefg	NBC11-03902	**3.61**	0.50	3.40	3.82	3	4
abcdefg	NBC11-04435	**3.61**	0.50	3.40	3.82	3	4
abcdefg	NBC11-05138	**3.60**	0.76	3.42	3.78	1	4
abcdefg	NBC11-02754	**3.59**	0.50	3.42	3.76	3	4
abcdefg	NBC11-04392	**3.59**	0.57	3.42	3.76	2	4
abcdefg	NBC11-04400	**3.59**	0.75	3.42	3.76	1	4
abcdefg	SP Banner	**3.59**	0.50	3.42	3.76	3	4
abcdefg	NBC11-03888	**3.59**	1.18	3.37	3.80	1	5
abcdefg	NBC11-04413	**3.59**	0.62	3.37	3.80	2	4
abcdefg	NBC11-04428	**3.56**	0.51	3.35	3.76	3	4
abcdefg	NBC11-04432	**3.56**	0.51	3.35	3.76	3	4
abcdefg	NBC11-02751	**3.52**	0.58	3.35	3.69	2	4
abcdefg	NBC11-04483	**3.52**	0.51	3.35	3.69	3	4
abcdefg	NBC11-03864	**3.50**	0.86	3.29	3.71	1	4
abcdefg	NBC11-04467	**3.50**	0.79	3.29	3.71	2	4
abcdefg	NBC11-04485	**3.50**	0.51	3.29	3.71	3	4
bcdefg	NBC11-02387	**3.48**	0.70	3.31	3.65	2	4
bcdefg	NBC11-03876	**3.48**	0.70	3.31	3.65	2	4
bcdefg	NBC11-03898	**3.47**	0.62	3.26	3.69	2	4
bcdefg	NBC11-03905	**3.47**	0.62	3.26	3.69	2	4
bcdefg	CARACAS	**3.44**	0.70	3.27	3.62	2	4
bcdefg	NBC11-02766	**3.44**	0.62	3.24	3.65	2	4
bcdefg	NBC11-03890	**3.44**	0.51	3.24	3.65	3	4
bcdefg	NBC11-03906	**3.44**	0.65	3.26	3.62	2	4
cdefg	NBC11-03827	**3.41**	0.80	3.24	3.58	1	4
defg	NBC11-02391	**3.37**	0.56	3.20	3.54	2	4
efg	NBC11-03823	**3.28**	0.68	3.10	3.46	2	4
fg	NBC11-02384	**3.26**	0.76	3.09	3.43	2	4
fg	NBC11-02752	**3.24**	0.66	3.02	3.45	2	4
g	Ag-Outback	**3.13**	1.19	2.94	3.31	1	4
g	NBC11-02757	**3.11**	0.83	2.90	3.32	2	4

Table A3. Mean days to flower measurement for all lines across both 2012 and 2013 field evaluations. Lines with the same significance letter code are not significantly different. The mean, standard deviation, lower confidence level (LCL), upper confidence level (UCL), minimum value (MIN) and maximum value (MAX) are reported.

significance	*NAME*	*MEAN*	*STDEV*	*LCL*	*UCL*	*MIN*	*MAX*
a	CARACAS	69.72	7.35	68.36	71.09	51	83
b	NBC11-02386	62.78	3.56	61.11	64.45	56	68
bc	NBC11-03890	60.75	6.65	58.98	62.52	45	69
bcd	NBC11-02752	60.18	6.24	58.46	61.89	47	70
bcde	NBC11-02389	59.06	4.93	57.34	60.78	47	69
bcde	NBC11-03893	58.89	3.01	57.22	60.56	54	63
bcde	NBC11-03906	58.24	6.09	57.02	59.45	49	70.5
bcde	NBC11-03879	58.21	5.50	57.03	59.39	49	72.5
bcde	NBC11-04483	58.19	5.04	57.01	59.38	49	70.5
bcdef	NBC11-04413	57.65	4.73	55.93	59.37	47	63
cdef	NBC11-04420	57.39	5.12	56.19	58.58	48	69.5

cdefg	NBC11-02757	57.31	5.76	55.54	59.08	47	64
cdefg	NBC11-04473	57.31	3.74	55.54	59.08	52	67
cdefg	NBC11-03823	57.26	4.60	56.05	58.48	50	67.5
cdefg	NBC11-04436	57.22	4.68	55.55	58.89	47	67
cdefg	NBC11-02751	57.01	7.30	55.83	58.19	46	74
cdefgh	NBC11-04485	56.94	5.15	55.27	58.61	48	68
cdefgh	NBC11-02387	56.58	5.36	55.40	57.76	48	68
cdefghi	NBC11-03837	56.44	4.77	54.77	58.11	48	65
cdefghi	NBC11-03903	56.22	4.28	54.55	57.89	45	62
cdefghi	NBC11-02753	55.96	4.99	54.76	57.15	48	66
defghi	NBC11-05148	55.69	5.34	54.51	56.88	47	65
defghij	NBC11-04418	55.61	2.52	53.94	57.28	54	65
defghij	NBC11-03898	55.59	3.26	53.87	57.31	52	65
defghijk	NBC11-04463	55.41	3.91	53.69	57.13	47	66
defghijk	NBC11-03844	55.33	10.29	53.66	57.00	41	68
efghijk	NBC11-02384	55.22	7.37	54.04	56.40	47	70.5
efghijk	NBC11-03864	55.17	5.42	53.50	56.84	43	70
efghijk	NBC11-04437	55.06	3.49	53.34	56.78	48	62
efghijk	NBC11-03842	55.06	5.66	53.86	56.25	48	67.5
efghijk	NBC11-02759	54.87	6.15	53.67	56.07	45	71.5
efghijk	NBC11-02400	54.85	6.11	53.67	56.03	44	67
efghijk	NBC11-04403	54.83	6.35	53.65	56.01	46	69.5
efghijk	NBC11-03902	54.72	3.14	53.05	56.39	50	61
efghijk	NBC11-04406	54.69	6.13	53.51	55.88	47	68
efghijk	NBC11-03827	54.64	7.03	53.46	55.82	46	68
efghijk	NBC11-02391	54.57	5.79	53.39	55.75	47	66.5
efghijkl	NBC11-03900	54.47	3.22	52.75	56.19	48	63
efghijkl	NBC11-04391	54.44	3.47	52.77	56.11	47	60
efghijkl	NBC11-02754	54.40	6.18	53.22	55.58	46	68
efghijkl	NBC11-02765	54.39	4.15	52.72	56.06	44	61
fghijkl	NBC11-04484	54.19	6.58	53.01	55.38	46	66
fghijklm	NBC11-03861	54.17	3.31	52.50	55.84	48	63
fghijklm	NBC11-03885	54.06	4.22	52.39	55.73	48	61
fghijklm	NBC11-04408	53.72	6.49	52.54	54.90	43	67
fghijklmn	NBC11-04428	53.72	3.25	52.05	55.39	50	61
fghijklmn	NBC11-05138	53.58	7.27	52.33	54.83	45	66
fghijklmn	NBC11-05135	53.56	6.62	52.36	54.75	47	66
fghijklmn	NBC11-04488	53.44	3.47	51.77	55.11	48	63
fghijklmno	NBC11-03874	53.39	2.43	51.72	55.06	49	57
fghijklmno	NBC11-02399	53.22	2.62	51.55	54.89	49	57
fghijklmno	NBC11-04392	52.97	7.15	51.79	54.15	44	66
fghijklmno	NBC11-03897	52.89	6.85	51.71	54.07	45	69
fghijklmnop	NBC11-04430	52.89	3.20	51.22	54.56	49	63
fghijklmnop	NBC11-03826	52.86	7.20	51.66	54.05	45	68.5
fghijklmnop	NBC11-03828	52.84	6.97	51.62	54.05	44	66.5
fghijklmnop	NBC11-03853	52.83	3.07	51.16	54.50	46	57
ghijklmnop	NBC11-05143	52.83	7.14	51.63	54.03	44	70.5
ghijklmnop	NBC11-04432	52.82	2.98	51.11	54.54	49	58
ghijklmnop	NBC11-03847	52.74	6.87	51.56	53.92	45	68.5
ghijklmnop	NBC11-04400	52.69	6.94	51.49	53.88	46	66
ghijklmnop	NBC11-05130	52.64	6.00	51.45	53.84	45	64
ghijklmnop	NBC11-03876	52.60	7.37	51.42	53.78	45	69
ghijklmnopq	NBC11-03835	52.56	2.28	50.89	54.23	49	56
hijklmnopq	NBC11-03718	52.33	5.91	51.15	53.51	44	63.5
hijklmnopq	NBC11-04435	52.17	2.38	50.50	53.84	49	56
hijklmnopq	NBC11-04444	52.17	3.63	50.50	53.84	48	60

hijklmnopq	NBC11-02401	52.11	2.27	50.44	53.78	49	55
ijklmnopq	NBC11-04384	52.11	5.84	50.93	53.29	43	64
ijklmnopq	NBC11-04474	52.00	2.98	50.28	53.72	48	59
ijklmnopq	NBC11-04454	51.93	6.55	50.75	53.11	43	64
ijklmnopq	AG-Outback	51.89	8.38	50.53	53.25	44	76.5
ijklmnopq	NBC11-02755	51.89	3.64	50.22	53.56	48	62
ijklmnopq	NBC11-02396	51.85	7.12	50.67	53.03	45	65
ijklmnopq	NBC11-03855	51.83	6.59	50.65	53.01	43	66
ijklmnopq	NBC11-03843	51.83	6.95	50.63	53.03	43	65.5
ijklmnopq	NBC11-02397	51.79	6.48	50.61	52.97	45	65
ijklmnopq	NBC11-03859	51.72	2.24	50.05	53.39	48	55
ijklmnopq	NBC11-03905	51.71	2.05	49.99	53.42	49	55
jklmnopq	NBC11-04462	51.32	6.26	50.14	52.50	44	64
jklmnopq	NBC11-05142	51.27	6.66	50.07	52.47	43	65
jklmnopqr	NBC11-04476	51.25	3.15	49.48	53.02	47	57
jklmnopqr	NBC11-03832	51.06	2.61	49.34	52.78	47	54
jklmnopqr	NBC11-02398	50.93	6.65	49.75	52.11	42	63
jklmnopqr	NBC11-04452	50.93	6.53	49.75	52.11	43	63.5
jklmnopqrs	NBC11-03884	50.83	2.48	49.16	52.50	48	55
jklmnopqrs	NBC11-04457	50.67	2.85	49.00	52.34	47	57
jklmnopqrs	NBC11-03833	50.61	2.70	48.94	52.28	48	55
jklmnopqrst	NBC11-02388	50.28	3.18	48.61	51.95	42	56
jklmnopqrst	NBC11-03838	50.24	2.75	48.52	51.95	46	55
klmnopqrst	NBC11-04456	50.06	2.71	48.39	51.73	46	54
klmnopqrst	NBC11-04389	50.00	2.45	48.33	51.67	46	54
lmnopqrst	NBC11-04434	50.00	5.91	48.82	51.18	42	63
lmnopqrst	NBC11-02766	49.94	3.32	48.27	51.61	45	56
lmnopqrst	NBC11-03854	49.94	3.42	48.22	51.66	46	57
lmnopqrst	SP Banner	49.90	6.71	48.72	51.08	43	63
lmnopqrst	NBC11-03822	49.61	3.57	47.94	51.28	44	57
mnopqrst	NBC11-04433	49.60	5.97	48.42	50.78	42	62.5
mnopqrst	NBC11-03845	49.47	3.48	47.75	51.19	43	56
mnopqrst	NBC11-04459	49.22	6.49	47.55	50.89	42	64
nopqrst	VT Barrier	49.17	7.77	47.99	50.35	41	64
nopqrst	NBC11-03717	49.06	2.60	47.39	50.73	46	53
nopqrst	NBC11-04396	48.76	2.73	47.05	50.48	45	54
nopqrst	NBC11-03856	48.72	2.24	47.05	50.39	46	53
opqrst	NBC11-05144	48.71	7.23	47.53	49.89	40	66
opqrst	NBC11-04467	48.67	5.46	47.00	50.34	42	57
opqrst	NBC11-04409	48.50	5.73	46.83	50.17	42	61
opqrst	NBC11-04487	48.35	2.64	46.63	50.07	45	53
opqrst	NBC11-04471	48.18	3.63	46.46	49.89	44	55
pqrst	NBC11-03869	47.67	2.61	46.00	49.34	45	54
pqrst	NBC11-03888	47.65	5.11	45.93	49.37	40	56
qrst	NBC11-03858	47.17	2.85	45.50	48.84	44	53
rst	NBC11-04479	46.00	3.07	44.33	47.67	42	52
rst	NBC11-04458	45.83	3.20	44.16	47.50	42	51
st	NBC11-02760	45.50	3.00	43.83	47.17	41	51
st	NBC11-03872	45.47	3.28	43.75	47.19	41	52
st	NBC11-02385	45.44	2.99	43.77	47.11	42	52
t	NBC11-04464	45.11	2.95	43.44	46.78	42	51
t	NBC11-04419	45.06	3.19	43.39	46.73	42	52

Table A4. Mean flowering period measurement for all lines across both 2012 and 2013 field evaluations. Lines with the same significance letter code are not significantly different. The mean, standard deviation, lower confidence level (LCL), upper confidence level (UCL), minimum value (MIN) and maximum value (MAX) are reported.

significance	*NAME*	*MEAN*	*STDEV*	*LCL*	*UCL*	*MIN*	*MAX*
a	NBC11-04444	**46.67**	5.32	44.70	48.63	36	53
ab	NBC11-04474	**44.94**	4.85	42.92	46.96	38	55
abc	NBC11-02766	**43.39**	7.04	41.42	45.35	28	54
abcd	NBC11-03853	**42.44**	4.57	40.48	44.41	36	50
abcd	NBC11-02765	**42.17**	3.76	40.20	44.13	33	48
abcde	NBC11-04463	**41.82**	5.78	39.80	43.85	30	51
abcdef	NBC11-04432	**40.41**	7.13	38.39	42.43	24	50
abcdefg	NBC11-02388	**40.29**	6.44	38.27	42.32	32	53
bcdefgh	NBC11-03833	**39.67**	5.96	37.70	41.63	29	49
bcdefghi	NBC11-04488	**39.28**	4.04	37.31	41.24	34	46
bcdefghi	NBC11-03874	**39.22**	4.63	37.26	41.19	32	50
bcdefghi	NBC11-04456	**39.17**	5.96	37.20	41.13	30	50
bcdefghi	NBC11-04396	**39.12**	5.17	37.09	41.14	31	49
bcdefghij	NBC11-03898	**38.53**	3.71	36.51	40.55	32	45
cdefghij	NBC11-04436	**38.44**	3.88	36.48	40.41	32	45
cdefghijk	NBC11-03905	**38.35**	5.96	36.33	40.38	31	51
cdefghijkl	NBC11-04413	**38.25**	4.60	36.16	40.34	31	47
cdefghijkl	NBC11-03838	**38.24**	5.17	36.21	40.26	30	48
cdefghijkl	NBC11-02386	**38.22**	4.82	36.26	40.19	26	49
cdefghijkl	NBC11-02755	**38.17**	3.31	36.20	40.13	33	43
cdefghijklm	NBC11-03893	**38.00**	4.13	36.03	39.97	31	47
cdefghijklmn	NBC11-04473	**37.81**	4.83	35.73	39.90	33	50
defghijklmn	NBC11-03826	**37.61**	6.17	36.15	39.06	27	49
defghijklmn	NBC11-02389	**37.59**	4.23	35.57	39.61	28	44
defghijklmn	NBC11-03859	**37.39**	5.79	35.42	39.35	29	52
defghijklmno	NBC11-03861	**37.35**	4.53	35.33	39.38	29	46
defghijklmno	NBC11-03845	**37.29**	5.75	35.27	39.32	25	47
defghijklmno	NBC11-03902	**37.22**	6.04	35.26	39.19	29	53
defghijklmno	NBC11-04485	**37.22**	4.63	35.26	39.19	28	45
defghijklmno	NBC11-03885	**37.17**	5.47	35.20	39.13	30	49
defghijklmnop	NBC11-04428	**37.06**	4.29	35.09	39.02	32	47
defghijklmnopq	NBC11-03903	**37.00**	7.39	34.98	39.02	15	47
defghijklmnopq	NBC11-04391	**36.89**	4.55	34.92	38.85	31	46
defghijklmnopqr	NBC11-03900	**36.71**	4.83	34.68	38.73	26	46
defghijklmnopqr	NBC11-04476	**36.69**	4.87	34.60	38.77	29	47
defghijklmnopqr	NBC11-02752	**36.47**	4.56	34.45	38.49	28	46
defghijklmnopqrs	NBC11-04437	**36.35**	6.43	34.33	38.38	26	48
efghijklmnopqrs	NBC11-05130	**36.33**	8.66	34.92	37.74	25.5	52
efghijklmnopqrst	CARACAS	**36.06**	5.49	34.04	38.08	20	43
efghijklmnopqrst	NBC11-03837	**35.89**	6.22	33.92	37.85	28	48
efghijklmnopqrstu	NBC11-02399	**35.78**	5.41	33.81	37.74	26	46
fghijklmnopqrstu	NBC11-04454	**35.58**	8.23	34.19	36.97	24	51
fghijklmnopqrstuv	NBC11-03864	**35.44**	5.88	33.48	37.41	26	46
fghijklmnopqrstuv	NBC11-03906	**35.40**	5.93	33.97	36.83	23	46
fghijklmnopqrstuv	NBC11-03854	**35.35**	6.51	33.33	37.38	25	51
fghijklmnopqrstuv	NBC11-03835	**35.33**	3.61	33.37	37.30	30	42
fghijklmnopqrstuv	NBC11-03856	**35.22**	7.09	33.26	37.19	26	49
fghijklmnopqrstuv	NBC11-03890	**35.19**	4.94	33.10	37.27	26	45
fghijklmnopqrstuv	NBC11-04406	**35.01**	5.57	33.62	36.40	26	49
fghijklmnopqrstuv	NBC11-02384	**34.99**	6.61	33.55	36.42	24	50
fghijklmnopqrstuvw	NBC11-02757	**34.88**	6.97	32.79	36.96	26	47

ghijklmnopqrstuvw	NBC11-04403	**34.71**	6.11	33.32	36.10	26	55
ghijklmnopqrstuvwx	NBC11-04487	**34.53**	8.32	32.51	36.55	21	53
ghijklmnopqrstuvwx	NBC11-03717	**34.50**	7.56	32.53	36.47	24	50
hijklmnopqrstuvwx	NBC11-03897	**34.46**	6.18	33.07	35.85	25.5	48
hijklmnopqrstuvwx	NBC11-04418	**34.44**	4.15	32.48	36.41	27	43
hijklmnopqrstuvwx	NBC11-04483	**34.17**	4.91	32.78	35.56	26.5	47
hijklmnopqrstuvwx	NBC11-05143	**34.16**	7.11	32.73	35.59	22	47
hijklmnopqrstuvwx	NBC11-05148	**34.15**	7.85	32.76	35.54	19	50
hijklmnopqrstuvwx	NBC11-04435	**34.11**	7.87	32.15	36.08	25	49
ijklmnopqrstuvwx	NBC11-02759	**34.07**	5.20	32.66	35.48	26	47
ijklmnopqrstuvwx	NBC11-03822	**34.06**	6.37	32.09	36.02	25	46
ijklmnopqrstuvwx	NBC11-03869	**33.89**	4.98	31.92	35.85	26	44
ijklmnopqrstuvwx	NBC11-03832	**33.82**	7.06	31.80	35.85	24	47
ijklmnopqrstuvwx	NBC11-04389	**33.67**	5.34	31.70	35.63	25	42
ijklmnopqrstuvwxy	NBC11-03884	**33.56**	5.07	31.59	35.52	23	45
ijklmnopqrstuvwxy	NBC11-02401	**33.33**	5.68	31.37	35.30	23	44
ijklmnopqrstuvwxy	NBC11-04457	**33.33**	5.80	31.37	35.30	25	47
jklmnopqrstuvwxy	NBC11-03823	**33.22**	5.34	31.79	34.65	23	47
jklmnopqrstuvwxy	NBC11-02754	**33.21**	7.21	31.82	34.60	22	52
jklmnopqrstuvwxy	NBC11-04392	**33.14**	7.10	31.75	34.53	24.5	50
jklmnopqrstuvwxyz	NBC11-03858	**33.06**	5.92	31.04	35.08	25	47
jklmnopqrstuvwxyz	NBC11-04484	**32.92**	5.60	31.53	34.31	24	44
klmnopqrstuvwxyz	NBC11-03879	**32.82**	6.15	31.43	34.21	22.5	49
klmnopqrstuvwxyz	NBC11-05138	**32.66**	7.14	31.16	34.16	24	48
lmnopqrstuvwxyz	NBC11-05135	**32.63**	6.01	31.20	34.06	21.5	46
lmnopqrstuvwxyz	NBC11-02751	**32.57**	6.01	31.18	33.96	22	47
mnopqrstuvwxyz	NBC11-02391	**32.49**	5.78	31.10	33.88	22.5	43
mnopqrstuvwxyz	NBC11-03827	**32.49**	6.77	31.10	33.88	22	48
nopqrstuvwxyz	NBC11-04408	**32.47**	6.91	31.08	33.86	24	48
nopqrstuvwxyz	NBC11-04400	**32.34**	5.10	30.93	33.75	24.5	41
nopqrstuvwxyz	NBC11-03828	**32.31**	6.19	30.88	33.74	23.5	49
nopqrstuvwxyz	NBC11-04420	**32.14**	4.65	30.73	33.55	25.5	44
nopqrstuvwxyz	NBC11-02397	**32.08**	5.75	30.69	33.47	24	49
opqrstuvwxyz	NBC11-02753	**31.76**	6.26	30.35	33.17	20	45
pqrstuvwxyz	NBC11-03847	**31.60**	5.97	30.21	32.99	21.5	46
pqrstuvwxyz	NBC11-02387	**31.58**	5.80	30.19	32.97	21.5	46
pqrstuvwxyzA	NBC11-04409	**31.56**	10.07	29.59	33.52	21	56
pqrstuvwxyzA	NBC11-03844	**31.53**	7.99	29.51	33.55	19	46
qrstuvwxyzA	NBC11-03718	**31.38**	7.52	29.98	32.77	22	50
rstuvwxyzA	NBC11-02396	**31.32**	6.08	29.93	32.71	22	45
rstuvwxyzA	NBC11-02400	**31.31**	4.94	29.92	32.70	24	43
rstuvwxyzAB	NBC11-04430	**31.28**	5.77	29.31	33.24	24	41
rstuvwxyzAB	NBC11-03843	**31.18**	6.40	29.75	32.61	22	54
rstuvwxyzAB	NBC11-04384	**31.11**	6.22	29.72	32.50	22	49
rstuvwxyzABC	NBC11-04467	**31.00**	9.14	29.03	32.97	20	48
stuvwxyzABC	NBC11-02398	**30.83**	6.26	29.44	32.22	23	48
stuvwxyzABC	NBC11-04471	**30.82**	7.23	28.80	32.85	24	44
stuvwxyzABC	NBC11-04459	**30.72**	9.83	28.76	32.69	20	51
tuvwxyzABC	NBC11-04452	**30.53**	5.86	29.14	31.92	23.5	45
tuvwxyzABC	NBC11-03842	**30.50**	6.93	29.09	31.91	21	49
uvwxyzABC	NBC11-05142	**30.30**	4.69	28.89	31.71	24	42
vwxyzABC	NBC11-04462	**30.24**	6.24	28.85	31.63	21	48
vwxyzABC	NBC11-03876	**29.99**	6.81	28.60	31.38	20	47
wxyzABC	NBC11-03855	**29.21**	6.72	27.82	30.60	19	48
xyzABC	NBC11-05144	**28.99**	7.41	27.58	30.40	18.5	47
yzABCD	NBC11-04434	**28.06**	5.40	26.67	29.45	21	43

zABCD	NBC11-04433	**27.47**	6.07	26.08	28.86	20	49
zABCD	NBC11-04464	**27.06**	9.16	25.09	29.02	20	60
zABCD	NBC11-03888	**26.82**	5.89	24.80	28.85	20	40
ABCD	AG-Outback	**26.10**	6.27	24.23	27.97	19	42
BCD	NBC11-04479	**25.06**	5.80	23.09	27.02	20	45
CD	NBC11-03872	**24.65**	6.33	22.62	26.67	19	43
D	VT Barrier	**24.33**	3.18	22.94	25.72	19	32
D	NBC11-04419	**24.22**	6.39	22.26	26.19	20	48
D	SP Banner	**23.96**	3.25	22.57	25.35	17	33
D	NBC11-04458	**23.78**	2.53	21.81	25.74	20	28
D	NBC11-02760	**23.39**	2.83	21.42	25.35	19	30
D	NBC11-02385	**23.28**	3.49	21.31	25.24	19	30

Table A5. Mean days to maturity measurement for all lines across both 2012 and 2013 field evaluations. Lines with the same significance letter code are not significantly different. The mean, standard deviation, lower confidence level (LCL), upper confidence level (UCL), minimum value (MIN) and maximum value (MAX) are reported.

significance	*NAME*	*MEAN*	*STDEV*	*LCL*	*UCL*	*MIN*	*MAX*
a	CARACAS	**121.75**	12.01	119.09	124.41	97	135
ab	NBC11-02386	**121.00**	6.59	118.25	123.75	111	131
abc	NBC11-04444	**120.50**	6.84	117.84	123.16	110	132
abcd	NBC11-04413	**120.00**	7.16	117.16	122.84	110	127
abcd	NBC11-02752	**119.73**	7.23	116.99	122.48	109	133
abcde	NBC11-04474	**119.29**	7.24	116.44	122.13	108	127
abcde	NBC11-04436	**119.13**	8.35	116.39	121.88	105	130
abcdef	NBC11-03893	**118.67**	7.03	115.92	121.41	109	130
abcdef	NBC11-04463	**118.53**	6.47	115.79	121.28	109	126
abcdefg	NBC11-02765	**118.27**	6.64	115.52	121.01	108	127
abcdefgh	NBC11-04473	**118.21**	6.93	115.37	121.06	108	127
abcdefgh	NBC11-02389	**118.07**	6.04	115.32	120.81	111	129
abcdefghi	NBC11-03890	**117.07**	9.63	114.32	119.81	95	127
abcdefghij	NBC11-04428	**116.60**	6.94	113.85	119.35	107	128
abcdefghijk	NBC11-03898	**116.50**	5.68	113.66	119.34	108	125
abcdefghijk	NBC11-04432	**116.36**	6.16	113.52	119.20	103	126
abcdefghijk	NBC11-03853	**116.27**	5.38	113.52	119.01	109	127
abcdefghijk	NBC11-04437	**116.27**	6.11	113.52	119.01	108	127
abcdefghijkl	NBC11-02757	**116.21**	7.33	113.37	119.06	105	130
abcdefghijkl	NBC11-03837	**116.20**	6.28	113.45	118.95	107	127
abcdefghijkl	NBC11-04488	**116.20**	6.60	113.45	118.95	106	127
abcdefghijkl	NBC11-03906	**116.19**	8.34	114.28	118.10	99	126
abcdefghijkl	NBC11-03903	**116.00**	10.89	113.34	118.66	92	130
abcdefghijkl	NBC11-04483	**115.92**	8.14	114.01	117.83	100	129
abcdefghijklm	NBC11-04485	**115.87**	7.12	113.12	118.61	104	127
abcdefghijklm	NBC11-03874	**115.73**	5.75	112.99	118.48	108	126
abcdefghijklm	NBC11-04420	**115.68**	7.87	113.74	117.62	98	127
abcdefghijklm	NBC11-03861	**115.64**	7.98	112.80	118.48	98	126
abcdefghijklm	NBC11-02388	**115.56**	8.26	112.90	118.22	103	129
abcdefghijklm	NBC11-04391	**115.53**	6.38	112.79	118.28	105	125
abcdefghijklm	NBC11-03879	**115.42**	8.35	113.48	117.36	99	128
abcdefghijklmn	NBC11-03900	**115.13**	8.69	112.39	117.88	97	126
abcdefghijklmn	NBC11-02384	**115.07**	8.78	113.13	117.01	101	127
abcdefghijklmn	NBC11-04403	**114.89**	7.69	112.98	116.80	98	127
abcdefghijklmno	NBC11-03902	**114.80**	6.91	112.05	117.55	100	126
abcdefghijklmno	NBC11-03823	**114.79**	7.62	112.88	116.70	99	126

abcdefghijklmnop	NBC11-03864	**114.67**	9.55	111.92	117.41	93	132
abcdefghijklmnopq	NBC11-03885	**114.60**	6.75	111.85	117.35	102	125
abcdefghijklmnopq	NBC11-02759	**114.55**	6.99	112.58	116.53	100	127
abcdefghijklmnopq	NBC11-04418	**114.47**	5.44	111.72	117.21	107	122
abcdefghijklmnopq	NBC11-02387	**114.38**	7.06	112.44	116.32	99	125
abcdefghijklmnopq	NBC11-05148	**114.34**	7.62	112.43	116.25	96	126.5
abcdefghijklmnopq	NBC11-05130	**114.33**	6.99	112.35	116.30	97	124.5
abcdefghijklmnopq	NBC11-03833	**114.27**	7.80	111.52	117.01	103	126
abcdefghijklmnopq	NBC11-02766	**114.25**	8.40	111.59	116.91	98	125
abcdefghijklmnopq	NBC11-03905	**114.13**	7.70	111.39	116.88	103	128
bcdefghijklmnopqr	NBC11-02751	**114.11**	7.78	112.20	116.02	99	127
bcdefghijklmnopqr	NBC11-04406	**114.00**	7.97	112.12	115.88	98	127
bcdefghijklmnopqr	NBC11-02753	**113.90**	7.52	111.92	115.87	96	124
bcdefghijklmnopqr	NBC11-03826	**113.70**	8.10	111.76	115.64	98	126
bcdefghijklmnopqr	NBC11-03859	**113.47**	6.03	110.89	116.05	107	125
cdefghijklmnopqr	NBC11-02391	**113.08**	7.14	111.17	114.99	98	126
cdefghijklmnopqr	NBC11-04454	**113.03**	7.92	111.09	114.97	96	127
cdefghijklmnopqr	NBC11-02399	**113.00**	6.00	110.25	115.75	105	125
defghijklmnopqr	NBC11-03827	**112.61**	8.55	110.70	114.52	99	126
defghijklmnopqr	NBC11-04408	**112.34**	8.54	110.46	114.22	98	126
defghijklmnopqr	NBC11-05135	**112.28**	7.30	110.40	114.16	98	124.5
defghijklmnopqr	NBC11-02754	**112.22**	8.73	110.28	114.16	97	126
defghijklmnopqrs	NBC11-02755	**112.20**	5.36	109.45	114.95	106	121
efghijklmnopqrs	NBC11-02400	**112.12**	7.46	110.27	113.97	96	124
efghijklmnopqrs	NBC11-05138	**111.97**	8.91	109.99	113.94	95	127
efghijklmnopqrs	NBC11-04456	**111.94**	6.60	109.28	114.60	103	126
efghijklmnopqrs	NBC11-04396	**111.93**	6.82	109.19	114.68	103	122
efghijklmnopqrs	NBC11-03838	**111.93**	6.96	109.09	114.77	103	123
efghijklmnopqrs	NBC11-04476	**111.86**	7.47	109.02	114.70	101	127
efghijklmnopqrs	NBC11-03897	**111.70**	8.34	109.82	113.58	99	125
fghijklmnopqrs	NBC11-04400	**111.44**	8.25	109.56	113.32	97	128
fghijklmnopqrs	NBC11-04484	**111.41**	8.47	109.59	113.24	97	128
fghijklmnopqrs	NBC11-04435	**111.38**	6.71	108.72	114.03	102	127
fghijklmnopqrs	NBC11-05143	**111.34**	8.46	109.46	113.22	97	128
fghijklmnopqrs	NBC11-04392	**111.17**	8.76	109.29	113.05	94	126
ghijklmnopqrs	NBC11-03842	**110.82**	7.24	108.97	112.67	98	126
ghijklmnopqrst	NBC11-03835	**110.75**	4.52	108.09	113.41	103	118
ghijklmnopqrst	NBC11-03847	**110.74**	8.66	108.91	112.56	96	125
ghijklmnopqrst	NBC11-03845	**110.50**	7.75	107.66	113.34	100	124
hijklmnopqrst	NBC11-03718	**110.48**	8.06	108.60	112.36	96	126
ijklmnopqrst	NBC11-02397	**110.06**	7.97	108.26	111.85	99	125
ijklmnopqrstu	NBC11-04457	**109.94**	6.98	107.28	112.60	99	125
jklmnopqrstu	NBC11-02396	**109.42**	9.34	107.57	111.27	97	125
jklmnopqrstuv	NBC11-04430	**109.18**	5.65	106.60	111.76	102	127
jklmnopqrstuv	NBC11-02401	**108.87**	6.14	106.12	111.61	98	121
klmnopqrstuv	NBC11-03828	**108.77**	9.30	106.92	110.62	94	128
klmnopqrstuv	NBC11-03832	**108.67**	6.17	105.92	111.41	101	122
klmnopqrstuv	NBC11-03854	**108.63**	5.92	105.97	111.28	102	120
klmnopqrstuv	NBC11-03884	**108.59**	6.42	106.01	111.17	102	125
lmnopqrstuv	NBC11-03855	**108.48**	6.78	106.63	110.34	96	122
lmnopqrstuv	NBC11-04452	**108.45**	8.38	106.60	110.31	95	125
mnopqrstuv	NBC11-04384	**108.35**	8.03	106.50	110.20	96	125
mnopqrstuv	NBC11-03717	**108.25**	6.77	105.59	110.91	100	122
nopqrstuv	NBC11-05142	**107.74**	7.76	105.91	109.56	95	122
nopqrstuvw	NBC11-03822	**107.65**	7.75	105.07	110.23	94	122
nopqrstuvw	NBC11-04389	**107.59**	4.50	105.01	110.17	100	117

nopqrstuvw	NBC11-03844	**107.44**	14.71	104.78	110.10	91	129
opqrstuvw	NBC11-03843	**107.28**	8.95	105.40	109.16	95	123
pqrstuvw	NBC11-04462	**107.08**	8.66	105.23	108.93	94	124
qrstuvw	NBC11-03876	**107.02**	9.36	105.14	108.90	94	122
rstuvw	NBC11-02398	**106.64**	8.75	104.79	108.49	94	122
rstuvwx	NBC11-04487	**106.53**	8.27	103.95	109.11	97	127
rstuvwxy	NBC11-03856	**106.40**	4.73	103.65	109.15	100	117
rstuvwxy	NBC11-03858	**105.67**	5.63	102.92	108.41	98	121
stuvwxy	NBC11-04433	**104.87**	8.17	103.04	106.69	93	124
stuvwxy	NBC11-05144	**104.78**	8.67	102.96	106.60	91	121
stuvwxyz	NBC11-03869	**104.53**	5.15	101.95	107.11	97	115
tuvwxyz	NBC11-04434	**103.97**	7.51	102.20	105.74	94	118
tuvwxyzA	NBC11-04467	**103.41**	10.88	100.83	105.99	92	124
tuvwxyzA	NBC11-04459	**103.00**	12.25	100.42	105.58	89	124
uvwxyzAB	NBC11-04409	**101.59**	11.03	99.01	104.17	88	124
vwxyzAB	NBC11-04471	**100.86**	7.89	98.02	103.70	94	122
vwxyzAB	NBC11-03888	**100.69**	9.48	98.03	103.35	91	120
wxyzAB	AG-Outback	**99.87**	10.08	97.43	102.31	89	128
xyzAB	SP Banner	**99.84**	7.95	98.05	101.64	91	119
yzAB	VT Barrier	**99.31**	7.70	97.53	101.08	87	113.5
zAB	NBC11-04479	**96.76**	7.44	94.19	99.34	88	122
zAB	NBC11-03872	**96.47**	6.18	93.89	99.05	89	113
zAB	NBC11-04464	**96.47**	3.84	93.89	99.05	90	104
AB	NBC11-04458	**96.11**	4.74	93.61	98.62	88	108
AB	NBC11-04419	**95.94**	6.34	93.44	98.45	88	116
AB	NBC11-02385	**95.39**	3.38	92.88	97.89	88	102
B	NBC11-02760	**94.33**	3.74	91.83	96.84	87	100

Table A6. Mean height ratings for all lines across both 2012 and 2013 field evaluations. Lines with the same significance letter code are not significantly different. The mean, standard deviation, lower confidence level (LCL), upper confidence level (UCL), minimum value (MIN) and maximum value (MAX) are reported.

significance	*NAME*	*MEAN*	*STDEV*	*LCL*	*UCL*	*MIN*	*MAX*
a	CARACAS	**4.97**	0.17	4.79	5.15	4	5
ab	NBC11-02386	**4.83**	0.38	4.58	5.09	4	5
abc	NBC11-04473	**4.50**	0.63	4.23	4.77	3	5
bc	NBC11-03906	**4.35**	0.60	4.17	4.54	3	5
bcd	NBC11-02765	**4.33**	0.69	4.08	4.59	3	5
bcd	NBC11-04436	**4.33**	0.69	4.08	4.59	3	5
bcde	NBC11-02389	**4.28**	0.67	4.03	4.53	3	5
bcde	NBC11-03893	**4.28**	0.57	4.03	4.53	3	5
bcde	NBC11-04483	**4.25**	0.73	4.07	4.43	2	5
bcdef	NBC11-04474	**4.24**	0.83	3.98	4.49	3	5
bcdef	NBC11-04413	**4.19**	0.66	3.92	4.46	3	5
bcdef	NBC11-05138	**4.18**	0.68	4.00	4.37	2	5
bcdef	NBC11-02388	**4.18**	0.39	3.92	4.44	4	5
bcdef	NBC11-03890	**4.18**	1.01	3.92	4.44	2	5
bcdef	NBC11-04437	**4.18**	0.53	3.92	4.44	3	5
bcdef	NBC11-03902	**4.17**	0.62	3.91	4.42	3	5
bcdef	NBC11-03903	**4.17**	0.86	3.91	4.42	2	5
bcdef	NBC11-04391	**4.17**	0.71	3.91	4.42	3	5
bcdefg	NBC11-02752	**4.12**	0.93	3.86	4.38	2	5
cdefg	NBC11-04406	**4.11**	0.62	3.93	4.29	2	5
cdefg	NBC11-04444	**4.11**	0.90	3.86	4.36	3	5
cdefg	NBC11-04484	**4.11**	0.67	3.93	4.29	3	5
cdefg	NBC11-02753	**4.09**	0.56	3.90	4.27	3	5
cdefg	NBC11-02387	**4.06**	0.71	3.88	4.23	3	5

cdefgh	NBC11-03864	**4.06**	0.80	3.80	4.31	3	5
cdefgh	NBC11-02751	**4.03**	0.56	3.85	4.21	3	5
cdefgh	NBC11-03823	**4.00**	0.74	3.82	4.18	2	5
cdefghi	NBC11-04428	**4.00**	0.49	3.75	4.25	3	5
cdefghi	NBC11-02384	**3.97**	0.51	3.79	4.15	3	5
cdefghij	NBC11-03844	**3.94**	1.16	3.69	4.20	2	5
cdefghij	NBC11-03853	**3.94**	0.64	3.69	4.20	3	5
cdefghij	NBC11-03885	**3.94**	0.54	3.69	4.20	3	5
cdefghij	NBC11-03900	**3.94**	0.83	3.68	4.20	2	5
cdefghij	NBC11-04432	**3.94**	0.75	3.68	4.20	3	5
cdefghij	NBC11-03879	**3.92**	0.77	3.74	4.10	2	5
cdefghij	NBC11-04420	**3.92**	0.73	3.74	4.10	2	5
cdefghij	NBC11-02759	**3.91**	0.66	3.73	4.10	2	5
cdefghij	NBC11-04418	**3.89**	0.76	3.64	4.14	3	5
cdefghij	NBC11-04463	**3.89**	0.76	3.64	4.14	3	5
cdefghij	NBC11-04488	**3.89**	0.76	3.64	4.14	2	5
cdefghij	NBC11-03898	**3.88**	0.78	3.62	4.14	3	5
cdefghij	NBC11-03859	**3.83**	0.51	3.58	4.09	3	5
cdefghij	NBC11-04408	**3.83**	0.56	3.65	4.01	3	5
cdefghij	NBC11-02391	**3.78**	0.68	3.60	3.96	2	5
cdefghij	NBC11-02400	**3.78**	0.42	3.60	3.96	3	4
cdefghij	NBC11-03897	**3.78**	0.64	3.60	3.96	2	5
cdefghij	NBC11-04400	**3.78**	0.64	3.60	3.96	2	5
cdefghij	NBC11-04403	**3.78**	0.64	3.60	3.96	3	5
cdefghijk	NBC11-04485	**3.78**	0.65	3.53	4.03	3	5
cdefghijk	NBC11-05143	**3.78**	0.59	3.60	3.96	3	5
cdefghijk	NBC11-05130	**3.77**	0.65	3.59	3.95	3	5
cdefghijkl	NBC11-03861	**3.76**	0.66	3.51	4.02	2	5
defghijkl	NBC11-05135	**3.74**	0.67	3.55	3.92	2	5
defghijkl	NBC11-02399	**3.72**	0.67	3.47	3.97	3	5
defghijkl	NBC11-02755	**3.72**	0.57	3.47	3.97	3	5
defghijkl	NBC11-03837	**3.72**	0.67	3.47	3.97	2	5
defghijkl	NBC11-03832	**3.71**	0.59	3.45	3.97	3	5
defghijkl	NBC11-03845	**3.71**	0.59	3.45	3.97	3	5
defghijkl	NBC11-03842	**3.69**	0.52	3.52	3.87	3	5
defghijkl	NBC11-03827	**3.67**	0.53	3.49	3.85	2	4
defghijkl	NBC11-03835	**3.67**	0.49	3.41	3.92	3	4
defghijkl	NBC11-03826	**3.66**	0.68	3.48	3.84	2	5
defghijkl	NBC11-02757	**3.65**	0.93	3.39	3.91	2	5
defghijkl	NBC11-03828	**3.65**	0.65	3.46	3.83	2	5
defghijkl	NBC11-03905	**3.65**	0.79	3.39	3.91	2	5
defghijkl	NBC11-02754	**3.64**	0.49	3.46	3.82	3	4
defghijkl	NBC11-05142	**3.63**	0.73	3.45	3.81	2	5
defghijklm	NBC11-04476	**3.63**	0.62	3.36	3.89	2	4
efghijklm	NBC11-02397	**3.61**	0.69	3.43	3.79	2	5
efghijklm	NBC11-03874	**3.61**	0.61	3.36	3.86	2	4
efghijklm	NBC11-04430	**3.61**	0.61	3.36	3.86	2	4
efghijklm	NBC11-04454	**3.61**	0.69	3.43	3.79	2	5
efghijklm	NBC11-05148	**3.61**	0.49	3.43	3.79	3	4
fghijklm	NBC11-03843	**3.56**	0.56	3.38	3.74	3	5
fghijklm	NBC11-03718	**3.56**	0.50	3.38	3.73	3	4
fghijklmn	NBC11-03822	**3.56**	0.78	3.30	3.81	2	5
fghijklmn	NBC11-04456	**3.56**	0.51	3.30	3.81	3	4
fghijklmn	NBC11-04396	**3.53**	0.51	3.27	3.79	3	4
fghijklmn	NBC11-02396	**3.53**	0.56	3.35	3.71	3	5
fghijklmn	NBC11-04452	**3.53**	0.51	3.35	3.71	3	4
fghijklmn	NBC11-02766	**3.50**	0.62	3.25	3.75	2	4
fghijklmn	NBC11-04389	**3.50**	0.71	3.25	3.75	2	5
fghijklmn	NBC11-04457	**3.50**	0.51	3.25	3.75	3	4
fghijklmn	NBC11-03717	**3.44**	0.70	3.19	3.70	2	5
fghijklmn	NBC11-03833	**3.44**	0.51	3.19	3.70	3	4
ghijklmn	NBC11-04392	**3.44**	0.56	3.27	3.62	3	5
hijklmn	NBC11-03847	**3.39**	0.55	3.21	3.57	2	4
hijklmn	NBC11-03855	**3.39**	0.49	3.21	3.57	3	4
hijklmn	NBC11-03884	**3.39**	0.61	3.14	3.64	2	4

hijklmn	NBC11-04435	**3.39**	0.61	3.14	3.64	2	4
hijklmn	NBC11-02398	**3.36**	0.49	3.18	3.54	3	4
hijklmn	NBC11-04462	**3.36**	0.64	3.18	3.54	2	5
ijklmn	NBC11-04384	**3.33**	0.53	3.15	3.51	2	4
ijklmn	NBC11-04434	**3.33**	0.63	3.15	3.51	2	5
ijklmn	NBC11-02401	**3.28**	0.67	3.03	3.53	2	4
ijklmn	NBC11-03856	**3.28**	0.57	3.03	3.53	2	4
jklmn	NBC11-03876	**3.28**	0.45	3.10	3.46	3	4
jklmn	NBC11-04459	**3.28**	0.83	3.03	3.53	2	5
jklmn	NBC11-04433	**3.25**	0.50	3.07	3.43	2	4
jklmn	NBC11-03838	**3.24**	0.56	2.98	3.49	2	4
jklmn	NBC11-03854	**3.24**	0.56	2.98	3.49	2	4
jklmn	NBC11-04409	**3.22**	0.73	2.97	3.47	2	5
jklmn	NBC11-04487	**3.18**	0.53	2.92	3.44	2	4
jklmn	NBC11-03858	**3.12**	0.49	2.86	3.38	2	4
jklmn	NBC11-03888	**3.12**	0.93	2.86	3.38	1	5
klmn	NBC11-05144	**3.11**	0.52	2.93	3.29	2	4
klmn	NBC11-04471	**3.06**	0.56	2.80	3.32	2	4
klmn	NBC11-02385	**3.06**	0.64	2.80	3.31	2	4
klmn	NBC11-03869	**3.06**	0.42	2.80	3.31	2	4
klmn	NBC11-04464	**3.06**	0.80	2.80	3.31	2	5
klmn	NBC11-04479	**3.06**	0.42	2.80	3.31	2	4
lmn	AG-Outback	**3.00**	0.60	2.78	3.22	2	4
lmn	NBC11-03872	**3.00**	0.61	2.74	3.26	2	4
lmn	NBC11-04419	**3.00**	0.49	2.75	3.25	2	4
lmn	NBC11-04467	**3.00**	0.49	2.75	3.25	2	4
lmn	NBC11-04458	**2.94**	0.54	2.69	3.20	2	4
mn	VT Barrier	**2.92**	0.37	2.74	3.10	2	4
mn	NBC11-02760	**2.89**	0.32	2.64	3.14	2	3
n	SP Banner	**2.86**	0.35	2.68	3.04	2	3

Table A7. Mean vigour ratings after the first frost for all lines across both 2012 and 2013 field evaluations. Lines with the same significance letter code are not significantly different. The mean, standard deviation, lower confidence level (LCL), upper confidence level (UCL), minimum value (MIN) and maximum value (MAX) are reported.

significance	*NAME*	*MEAN*	*STDEV*	*LCL*	*UCL*	*MIN*	*MAX*
a	NBC11-04409	**4.08**	0.28	3.76	4.40	4	5
a	NBC11-04464	**4.08**	0.28	3.76	4.40	4	5
ab	NBC11-02385	**4.00**	0.38	3.70	4.30	3	5
abc	NBC11-02760	**4.00**	0.00	3.65	4.35	4	4
abc	NBC11-03844	**4.00**	0.00	3.68	4.32	4	4
abc	NBC11-03888	**4.00**	0.00	3.69	4.31	4	4
abc	NBC11-04419	**4.00**	0.00	3.68	4.32	4	4
abc	NBC11-04459	**3.93**	0.27	3.62	4.24	3	4
abc	NBC11-03872	**3.88**	0.34	3.59	4.16	3	4
abc	NBC11-03890	**3.87**	0.35	3.57	4.16	3	4
abc	NBC11-04458	**3.86**	0.36	3.55	4.16	3	4
abc	NBC11-04444	**3.81**	0.40	3.52	4.10	3	4
abcd	NBC11-04413	**3.75**	0.45	3.42	4.08	3	4
abcd	NBC11-03858	**3.71**	0.47	3.41	4.02	3	4
abcd	NBC11-02399	**3.71**	0.47	3.43	3.99	3	4
abcd	NBC11-04463	**3.67**	0.62	3.37	3.96	2	4
abcde	NBC11-02765	**3.62**	0.51	3.30	3.93	3	4
abcde	NBC11-03718	**3.62**	0.51	3.30	3.93	3	4
abcde	NBC11-04479	**3.62**	0.51	3.30	3.93	3	4
abcde	SP Banner	**3.60**	0.51	3.30	3.90	3	4
abcde	NBC11-03903	**3.58**	0.51	3.25	3.92	3	4
abcde	NBC11-04437	**3.54**	0.52	3.22	3.86	3	4
abcde	NBC11-03861	**3.53**	0.64	3.24	3.83	2	4
abcde	NBC11-04488	**3.53**	0.52	3.24	3.83	3	4
abcde	NBC11-04454	**3.52**	0.60	3.27	3.78	2	4

abcde	NBC11-03845	**3.50**	0.63	3.21	3.79	2	4
abcde	NBC11-03853	**3.50**	0.52	3.19	3.81	3	4
abcde	NBC11-03900	**3.50**	0.52	3.19	3.81	3	4
abcde	NBC11-04474	**3.50**	0.52	3.17	3.83	3	4
abcde	NBC11-05130	**3.48**	0.79	3.24	3.72	1	5
abcde	NBC11-02755	**3.47**	0.77	3.21	3.74	2	4
abcde	NBC11-03822	**3.47**	0.61	3.21	3.74	2	4
abcde	NBC11-02386	**3.46**	0.66	3.14	3.78	2	4
abcde	NBC11-03893	**3.46**	0.78	3.14	3.78	2	4
abcde	NBC11-03832	**3.44**	0.70	3.17	3.72	2	4
abcde	NBC11-02389	**3.42**	0.79	3.08	3.75	2	4
abcde	NBC11-03906	**3.39**	0.72	3.15	3.63	2	4
abcde	NBC11-04462	**3.39**	0.84	3.15	3.63	2	5
abcde	NBC11-02397	**3.38**	0.59	3.13	3.63	2	4
abcde	NBC11-03864	**3.38**	0.81	3.09	3.66	2	4
abcde	NBC11-02400	**3.37**	0.76	3.10	3.63	2	4
abcde	NBC11-04384	**3.35**	1.07	3.11	3.59	1	5
abcde	NBC11-02401	**3.33**	0.91	3.08	3.58	1	4
abcde	NBC11-03826	**3.33**	0.91	3.06	3.60	1	4
abcde	NBC11-03833	**3.33**	0.73	3.08	3.58	2	4
abcde	NBC11-03855	**3.33**	0.80	3.08	3.58	2	4
abcde	NBC11-03869	**3.33**	0.80	3.08	3.58	2	4
abcde	NBC11-04392	**3.33**	1.06	3.08	3.58	1	4
abcde	NBC11-04396	**3.33**	0.97	3.08	3.58	1	4
abcde	NBC11-04487	**3.33**	0.76	3.10	3.57	2	4
abcde	NBC11-05142	**3.33**	0.91	3.08	3.58	1	5
abcde	NBC11-03847	**3.32**	0.84	3.07	3.56	1	4
abcde	NBC11-02759	**3.32**	0.67	3.05	3.58	2	4
abcde	NBC11-05148	**3.32**	0.67	3.05	3.58	2	4
abcde	NBC11-03835	**3.31**	0.63	2.99	3.63	2	4
abcde	NBC11-02753	**3.29**	0.95	3.06	3.53	1	4
abcde	NBC11-02384	**3.29**	0.90	3.03	3.54	1	5
abcde	NBC11-02391	**3.29**	0.61	2.98	3.59	2	4
abcde	NBC11-04435	**3.29**	0.90	3.03	3.54	1	4
abcde	NBC11-03717	**3.25**	0.45	2.92	3.58	3	4
abcde	NBC11-04484	**3.24**	0.77	2.99	3.49	2	4
abcde	NBC11-03823	**3.23**	0.73	2.91	3.55	2	4
abcde	NBC11-03902	**3.23**	0.44	2.91	3.55	3	4
abcde	NBC11-03828	**3.23**	0.92	2.98	3.47	1	4
abcde	CARACAS	**3.22**	0.55	2.95	3.49	2	4
abcde	NBC11-05144	**3.22**	0.85	2.98	3.46	1	4
abcde	NBC11-04403	**3.21**	1.08	2.95	3.47	1	5
abcde	NBC11-03827	**3.20**	0.77	2.94	3.46	2	4
abcde	NBC11-04406	**3.20**	0.77	2.94	3.46	2	4
abcde	NBC11-05135	**3.20**	0.62	2.94	3.46	2	4
abcde	NBC11-04456	**3.18**	1.05	2.94	3.43	1	4
abcde	NBC11-04452	**3.17**	1.15	2.93	3.41	1	4
abcde	NBC11-03843	**3.17**	0.70	2.93	3.40	2	4
abcde	NBC11-04389	**3.16**	0.83	2.89	3.42	1	4
abcde	NBC11-02766	**3.15**	0.80	2.83	3.47	2	4
abcde	NBC11-03837	**3.15**	0.69	2.83	3.47	2	4
abcde	NBC11-03854	**3.15**	0.81	2.89	3.41	1	4
abcde	NBC11-04418	**3.15**	0.59	2.89	3.41	2	4
bcde	NBC11-02751	**3.14**	1.01	2.89	3.39	1	4
bcde	NBC11-03884	**3.14**	0.91	2.89	3.39	1	4
bcde	NBC11-05138	**3.14**	0.91	2.89	3.39	1	4
bcde	NBC11-04483	**3.14**	0.83	2.89	3.38	1	4
bcde	NBC11-04400	**3.13**	0.92	2.89	3.37	1	5
cde	NBC11-03885	**3.13**	0.90	2.89	3.36	1	4
cde	NBC11-02752	**3.11**	0.78	2.73	3.50	2	4
cde	NBC11-03859	**3.11**	0.96	2.84	3.38	1	4
cde	NBC11-02398	**3.10**	1.07	2.84	3.36	1	4
cde	NBC11-03897	**3.10**	0.91	2.84	3.36	1	4
cde	NBC11-04420	**3.10**	0.91	2.84	3.36	1	4
cde	NBC11-04433	**3.10**	0.70	2.84	3.35	1	4

cde	NBC11-03856	**3.09**	0.85	2.85	3.33	1	4
cde	NBC11-04432	**3.08**	0.29	2.75	3.42	3	4
cde	NBC11-03874	**3.07**	0.47	2.76	3.38	2	4
cde	NBC11-04436	**3.07**	0.80	2.77	3.36	2	4
cde	NBC11-03905	**3.06**	0.90	2.78	3.34	1	4
cde	NBC11-03838	**3.06**	0.80	2.78	3.33	1	4
cde	NBC11-03842	**3.06**	1.16	2.78	3.33	1	4
cde	NBC11-04457	**3.06**	0.87	2.78	3.33	1	4
cde	NBC11-02757	**3.05**	0.83	2.79	3.31	1	4
cde	NBC11-02388	**3.05**	0.80	2.80	3.30	2	4
cde	NBC11-04408	**3.05**	0.80	2.80	3.30	1	4
cde	NBC11-04476	**3.05**	0.97	2.80	3.30	1	4
de	VT Barrier	**3.05**	0.87	2.90	3.19	1	4
de	NBC11-02754	**3.05**	0.79	2.80	3.29	1	4
de	NBC11-02387	**3.04**	0.82	2.80	3.28	1	4
de	Ag-Outback	**3.00**	0.79	2.72	3.28	1	4
de	NBC11-02396	**3.00**	0.86	2.74	3.26	1	4
de	NBC11-03898	**3.00**	0.91	2.73	3.27	1	4
de	NBC11-04391	**3.00**	0.55	2.69	3.31	2	4
de	NBC11-04430	**3.00**	0.65	2.74	3.26	2	4
de	NBC11-04434	**3.00**	0.80	2.76	3.24	1	4
de	NBC11-04471	**3.00**	0.87	2.75	3.25	1	4
de	NBC11-04473	**3.00**	0.43	2.67	3.33	2	4
de	NBC11-05143	**2.95**	1.03	2.68	3.21	1	4
de	NBC11-03876	**2.94**	0.80	2.67	3.22	1	4
de	NBC11-04467	**2.90**	0.32	2.54	3.26	2	3
de	NBC11-04485	**2.82**	0.88	2.54	3.10	1	4
de	NBC11-03879	**2.81**	0.91	2.52	3.10	1	4
e	NBC11-04428	**2.79**	0.78	2.56	3.03	1	4

Table A8. Mean vigour ratings after the second frost for all lines across both 2012 and 2013 field evaluations. Lines with the same significance letter code are not significantly different. The mean, standard deviation, lower confidence level (LCL), upper confidence level (UCL), minimum value (MIN) and maximum value (MAX) are reported.

significance	*NAME*	*MEAN*	*STDEV*	*LCL*	*UCL*	*MIN*	*MAX*
a	NBC11-04459	**3.93**	0.26	3.69	4.18	3	4
a	NBC11-04458	**3.93**	0.27	3.68	4.18	3	4
a	NBC11-04419	**3.92**	0.28	3.66	4.18	3	4
ab	NBC11-02760	**3.91**	0.30	3.62	4.19	3	4
ab	NBC11-02385	**3.88**	0.34	3.64	4.11	3	4
ab	NBC11-03890	**3.87**	0.35	3.62	4.11	3	4
ab	NBC11-03888	**3.86**	0.36	3.61	4.11	3	4
ab	NBC11-03718	**3.85**	0.38	3.58	4.11	3	4
ab	NBC11-03844	**3.85**	0.38	3.58	4.11	3	4
ab	NBC11-04409	**3.85**	0.55	3.58	4.11	2	4
ab	NBC11-04474	**3.83**	0.39	3.56	4.11	3	4
abc	NBC11-03858	**3.77**	0.44	3.51	4.03	3	4
abc	NBC11-04464	**3.77**	0.44	3.51	4.03	3	4
abc	NBC11-03872	**3.75**	0.45	3.51	3.99	3	4
abcd	NBC11-04413	**3.75**	0.45	3.48	4.02	3	4
abcd	NBC11-04432	**3.75**	0.45	3.48	4.02	3	4
abcd	SP Banner	**3.73**	0.46	3.49	3.98	3	4
abcd	NBC11-02399	**3.69**	0.48	3.45	3.92	3	4
abcd	NBC11-03855	**3.67**	0.77	3.44	3.89	1	4
abcde	NBC11-04488	**3.67**	0.49	3.42	3.91	3	4
abcde	CARACAS	**3.64**	0.63	3.39	3.89	2	4
abcde	NBC11-02765	**3.64**	0.50	3.39	3.89	3	4
abcde	NBC11-03845	**3.64**	0.50	3.39	3.89	3	4
abcde	NBC11-03861	**3.60**	0.51	3.36	3.84	3	4
abcde	NBC11-04444	**3.60**	0.51	3.36	3.84	3	4
abcde	NBC11-04437	**3.58**	0.51	3.31	3.86	3	4

abcde	NBC11-03906	**3.55**	0.60	3.34	3.76	2	4
abcde	NBC11-03902	**3.54**	0.52	3.28	3.80	3	4
abcde	NBC11-04479	**3.54**	0.52	3.28	3.80	3	4
abcde	NBC11-04391	**3.53**	0.52	3.29	3.78	3	4
abcde	NBC11-04463	**3.53**	0.52	3.29	3.78	3	4
abcde	NBC11-02388	**3.53**	0.62	3.30	3.76	2	4
abcde	NBC11-04456	**3.53**	0.62	3.30	3.76	2	4
abcde	NBC11-05130	**3.53**	0.51	3.31	3.74	3	4
abcde	NBC11-02396	**3.50**	0.52	3.26	3.74	3	4
abcde	NBC11-02397	**3.50**	0.62	3.28	3.72	2	4
abcde	NBC11-03864	**3.50**	0.52	3.26	3.74	3	4
abcde	NBC11-03903	**3.50**	0.52	3.23	3.77	3	4
abcde	NBC11-04454	**3.50**	0.71	3.28	3.72	2	4
abcde	NBC11-04384	**3.47**	0.70	3.26	3.69	2	4
abcde	NBC11-03833	**3.47**	0.94	3.24	3.70	1	4
abcde	NBC11-04420	**3.47**	0.72	3.24	3.70	2	4
abcde	NBC11-05148	**3.47**	0.62	3.24	3.70	2	4
abcde	NBC11-02759	**3.47**	0.74	3.22	3.71	2	4
abcde	NBC11-04457	**3.47**	0.52	3.22	3.71	3	4
abcde	NBC11-03717	**3.46**	0.66	3.20	3.72	2	4
abcde	NBC11-03823	**3.46**	0.52	3.20	3.72	3	4
abcde	NBC11-02389	**3.45**	0.52	3.17	3.74	3	4
abcde	NBC11-02752	**3.44**	0.53	3.13	3.76	3	4
abcde	NBC11-03843	**3.44**	0.62	3.22	3.67	2	4
abcde	NBC11-04396	**3.44**	0.70	3.22	3.67	2	4
abcde	NBC11-03832	**3.44**	0.63	3.20	3.67	2	4
abcde	NBC11-03893	**3.42**	0.51	3.14	3.69	3	4
abcde	NBC11-02753	**3.40**	0.60	3.19	3.61	2	4
abcde	NBC11-02754	**3.39**	0.78	3.17	3.61	1	4
abcde	NBC11-03874	**3.38**	0.51	3.12	3.65	3	4
abcde	NBC11-02384	**3.38**	0.72	3.14	3.61	2	4
abcde	NBC11-03822	**3.38**	0.72	3.14	3.61	2	4
abcde	NBC11-04389	**3.38**	0.62	3.14	3.61	2	4
abcde	NBC11-04483	**3.38**	0.62	3.14	3.61	2	4
abcde	NBC11-02387	**3.37**	0.68	3.15	3.58	2	4
abcde	NBC11-02386	**3.36**	0.50	3.11	3.61	3	4
abcde	NBC11-03853	**3.36**	0.63	3.11	3.61	2	4
abcde	NBC11-04436	**3.33**	0.49	3.09	3.58	3	4
abcde	NBC11-04473	**3.33**	0.49	3.06	3.61	3	4
abcde	NBC11-05135	**3.33**	0.77	3.11	3.56	2	4
abcde	NBC11-04462	**3.32**	0.89	3.10	3.53	1	4
abcde	NBC11-05144	**3.32**	0.82	3.10	3.53	1	4
abcde	NBC11-04392	**3.31**	1.08	3.08	3.55	1	4
abcde	NBC11-04418	**3.31**	0.79	3.08	3.55	2	4
abcde	NBC11-04435	**3.31**	0.79	3.08	3.55	2	4
abcde	NBC11-05142	**3.31**	0.70	3.08	3.55	2	4
abcde	NBC11-02766	**3.31**	0.48	3.05	3.57	3	4
abcde	NBC11-03835	**3.31**	0.48	3.05	3.57	3	4
abcde	NBC11-03837	**3.31**	0.63	3.05	3.57	2	4
abcde	NBC11-03856	**3.30**	0.80	3.09	3.51	1	4
abcde	NBC11-03827	**3.29**	0.85	3.07	3.52	1	4
abcde	NBC11-05138	**3.29**	1.05	3.07	3.52	1	4
abcde	NBC11-02391	**3.29**	0.61	3.03	3.54	2	4
abcde	NBC11-03900	**3.29**	0.61	3.03	3.54	2	4
abcde	NBC11-03847	**3.28**	0.57	3.06	3.50	2	4
abcde	NBC11-03869	**3.28**	0.75	3.06	3.50	2	4
abcde	NBC11-04430	**3.28**	0.75	3.06	3.50	1	4
abcde	NBC11-04471	**3.28**	0.83	3.06	3.50	1	4
abcde	NBC11-03838	**3.27**	1.03	3.02	3.51	1	4
abcde	NBC11-04403	**3.27**	0.70	3.02	3.51	2	4
abcde	NBC11-03885	**3.26**	0.65	3.05	3.48	2	4
abcde	NBC11-04400	**3.26**	0.81	3.05	3.48	1	4
abcde	NBC11-04452	**3.26**	0.99	3.05	3.48	1	4
abcde	NBC11-03859	**3.25**	0.45	3.01	3.49	3	4
abcde	NBC11-04434	**3.25**	0.79	3.04	3.46	2	4

abcde	NBC11-04487	**3.25**	0.72	3.04	3.46	1	4
abcde	NBC11-02398	**3.24**	0.97	3.01	3.46	1	4
abcde	NBC11-03897	**3.24**	0.75	3.01	3.46	1	4
abcde	NBC11-04406	**3.24**	0.66	3.01	3.46	2	4
abcde	NBC11-04433	**3.24**	0.75	3.01	3.46	2	4
abcde	NBC11-04476	**3.24**	0.90	3.01	3.46	1	4
bcde	VT Barrier	**3.21**	0.81	3.06	3.36	1	4
bcde	NBC11-02400	**3.20**	0.68	2.96	3.44	2	4
bcde	NBC11-03876	**3.20**	0.68	2.96	3.44	2	4
bcde	NBC11-03898	**3.20**	0.77	2.96	3.44	2	4
bcde	NBC11-03905	**3.20**	0.56	2.96	3.44	2	4
bcde	NBC11-05143	**3.20**	0.56	2.96	3.44	2	4
bcde	NBC11-02755	**3.19**	0.54	2.95	3.42	2	4
bcde	NBC11-02401	**3.18**	0.81	2.95	3.41	1	4
bcde	NBC11-03854	**3.18**	0.53	2.95	3.41	2	4
bcde	NBC11-03884	**3.18**	0.73	2.95	3.41	1	4
bcde	NBC11-02751	**3.17**	0.79	2.94	3.39	1	4
bcde	NBC11-04484	**3.17**	0.99	2.94	3.39	1	4
bcde	NBC11-04428	**3.16**	0.69	2.94	3.37	2	4
bcde	NBC11-03828	**3.15**	0.81	2.94	3.36	1	4
bcde	NBC11-03826	**3.14**	0.95	2.89	3.39	1	4
bcde	NBC11-04485	**3.14**	0.77	2.89	3.39	2	4
bcde	NBC11-04408	**3.13**	0.81	2.89	3.36	1	4
bcde	NBC11-03842	**3.07**	1.03	2.82	3.31	1	4
cde	Ag-Outback	**3.00**	1.03	2.76	3.24	1	4
cde	NBC11-03879	**3.00**	0.74	2.73	3.27	2	4
de	NBC11-02757	**2.94**	0.68	2.70	3.17	2	4
e	NBC11-04467	**2.80**	0.63	2.50	3.10	2	4

Appendix II: ANOVA Tables

Table A9. ANOVA results for days to flower. Data across repetitions, locations and years were utilized in this analysis.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genotype	118	38361	325	24.910	< 2e-16 ***
bloc	5	24656	4931	377.861	< 2e-16 ***
location	2	5030	2515	192.717	< 2e-16 ***
year	1	14599	14599	1118.618	< 2e-16 ***
location:year	2	3960	1980	151.736	< 2e-16 ***
bloc:location	10	712	71	5.458	4.71e-08 ***
genotype:location	236	4235	18	1.375	0.000253 ***
genotype:year	48	2085	43	3.329	2.23e-13 ***
genotype:location:year	96	667	7	0.532	0.999942
Residuals	2439	31830	13		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table A10. ANOVA results for days of flowering. Data across repetitions, locations and years were utilized in this analysis.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genotype	118	50912	431	23.849	< 2e-16 ***
bloc	5	1527	305	16.884	< 2e-16 ***
location	2	28220	14110	779.944	< 2e-16 ***
year	1	8959	8959	495.194	< 2e-16 ***
location:year	2	1115	557	30.814	6.11e-14 ***
bloc:location	10	1365	136	7.543	6.24e-12 ***
genotype:location	236	15213	64	3.563	< 2e-16 ***
genotype:year	47	1751	37	2.059	3.49e-05 ***
genotype:location:year	93	2100	23	1.248	0.0568 .
Residuals	2411	43618	18		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table A11. ANOVA results for days to maturity. Data across repetitions, locations and years were utilized in this analysis.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genotype	118	84866	719	24.471	< 2e-16 ***
bloc	5	29939	5988	203.735	< 2e-16 ***
location	2	19257	9629	327.606	< 2e-16 ***
year	1	5885	5885	200.241	< 2e-16 ***
location:year	2	3157	1579	53.715	< 2e-16 ***
bloc:location	10	13146	1315	44.728	< 2e-16 ***
genotype:location	236	14248	60	2.054	< 2e-16 ***
genotype:year	48	4579	95	3.246	9.99e-13 ***
genotype:location:year	92	1771	19	0.655	0.995
Residuals	2112	62073	29		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table A12. ANOVA results for plant height. Data across repetitions, locations and years were utilized in this analysis.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genotype	118	486.6	4.12	13.832	< 2e-16 ***
bloc	5	9.7	1.93	6.475	5.47e-06 ***
location	2	86.8	43.39	145.551	< 2e-16 ***
year	1	56.9	56.86	190.709	< 2e-16 ***
location:year	2	9.2	4.60	15.446	2.16e-07 ***
bloc:location	10	32.7	3.27	10.956	< 2e-16 ***
genotype:location	236	117.0	0.50	1.663	6.48e-09 ***
genotype:year	48	23.9	0.50	1.667	0.00289 **
genotype:location:year	95	42.4	0.45	1.496	0.00161 **
Residuals	2447	729.5	0.30		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table A13. ANOVA results for early plant vigour. Data across repetitions, locations and years were utilized in this analysis.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genotype	118	82.6	0.700	3.425	< 2e-16 ***
bloc	5	9.2	1.844	9.023	1.71e-08 ***
location	2	12.1	6.050	29.611	2.11e-13 ***
year	1	27.4	27.432	134.259	< 2e-16 ***
location:year	2	37.4	18.720	91.622	< 2e-16 ***
bloc:location	10	42.5	4.255	20.823	< 2e-16 ***
genotype:location	236	90.5	0.383	1.876	8.89e-13 ***
genotype:year	48	54.0	1.125	5.504	< 2e-16 ***
genotype:location:year	95	34.6	0.364	1.780	8.84e-06 ***
Residuals	2034	415.6	0.204		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table A14. ANOVA results for plant vigour after the first frost. Data across repetitions, locations and years were utilized in this analysis.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genotype	118	92.9	0.79	3.411	< 2e-16 ***
bloc	8	61.7	7.71	33.390	< 2e-16 ***
location	1	185.2	185.15	800.865	< 2e-16 ***
year	1	114.0	114.02	493.817	< 2e-16 ***
bloc:location	8	19.7	2.46	10.660	1.12e-14 ***
genotype:location	118	36.7	0.31	1.347	0.00957 **
genotype:year	72	36.8	0.51	2.214	4.94e-08 ***
Residuals	1553	358.6	0.23		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table A15. ANOVA table for plant vigour after the second frost. Data across repetitions, locations and years were utilized in this analysis.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genotype	118	162.1	1.37	3.985	< 2e-16 ***
bloc	8	84.6	10.57	30.660	< 2e-16 ***
location	1	32.5	32.53	94.331	< 2e-16 ***
year	1	289.2	289.17	838.621	< 2e-16 ***
location:year	1	33.4	33.39	96.841	< 2e-16 ***
bloc:location	8	67.1	8.39	24.330	< 2e-16 ***
genotype:location	118	52.4	0.44	1.288	0.0232 *
genotype:year	72	33.2	0.46	1.335	0.0336 *
genotype:location:year	72	30.5	0.42	1.229	0.0960 .
Residuals	1766	608.9	0.34		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table A16. ANOVA table for the 2012 cold germination experiment at 1 ºC. Data across repetitions were utilized in this analysis.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genotype	7	15.364	2.195	9.82	0.0037 **
bloc	1	0.051	0.051	0.23	0.6486
Residuals	7	1.564	0.223		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table A17. ANOVA table for the 2012 cold germination experiment at 4 ºC. Data across repetitions were utilized in this analysis.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genotype	7	4350.274	621.468	2.21	0.1581
bloc	1	271.426	271.426	0.97	0.3582
Residuals	7	1964.919	280.703		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table A18. ANOVA table for the 2012 cold germination experiment at 22 ºC. Data across repetitions were utilized in this analysis.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genotype	7	14660.788	2094.398	527.98	0.0000 ***
bloc	1	2.103	2.103	0.53	0.4902
Residuals	7	27.768	3.967		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table A19. ANOVA table for the 2013 cold germination experiment at 4 ºC. Data across repetitions were utilized in this analysis.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genotype	11	3533.833	321.258	7.35	0.0013 *
bloc	1	1.500	1.500	0.03	0.8564
Residuals	11	480.500	43.682		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table A20. ANOVA table for the 2013 cold germination experiment at 12 ºC. Data across repetitions were utilized in this analysis.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genotype	11	1550.000	140.909	7.24	0.0014 *
bloc	1	54.000	54.000	2.78	0.1239
Residuals	11	214.000	19.455		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table A21. ANOVA table for the 2013 cold germination experiment at 20 ºC. Data across repetitions were utilized in this analysis.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
genotype	11	1289.333	117.212	13.25	0.0001 **
bloc	1	2.667	2.667	0.30	0.5940
Residuals	11	97.333	8.848		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix III: Adaptation Summary

Table A22. Means of six selected elite DH lines, the two parental lines and two check cultivars for six agronomic traits of interest. “VT”, “SP”, “C” or “AO” indicates that mean is not statistically different from that check or parental mean at p = 0.05.

Name	Frost Vigour	Early Vigour	Days to Flower	Flowering Period	Days to Maturity	Height
NBC11-02385	3.88^{VT,SP,C}	4.00^{VT,SP,C}	45.4^{VT,SP}	23.3^{VT,SP,AO}	95.4^{VT,SP,AO}	3.06^{VT,SP,AO}
NBC11-02760	3.91^{VT,SP,C}	3.94^{VT,SP,C}	45.5^{VT,SP}	23.4^{VT,SP,AO}	94.3^{VT,SP,AO}	2.89^{VT,SP,AO}
NBC11-03888	3.86^{VT,SP,C}	3.59^{VT,SP,C,AO}	47.6^{VT,SP,AO}	26.8^{VT,SP,AO}	100.7^{VT,SP,AO}	3.12^{VT,SP,AO}
NBC11-04419	3.92^{SP,C}	4.00^{VT,SP,C}	45.1^{VT,SP}	24.2^{VT,SP,AO}	95.9^{VT,SP,AO}	3.00^{VT,SP,AO}
NBC11-04458	3.93^{SP,C}	4.06^{VT,SP,C}	45.8^{VT,SP}	23.8^{VT,SP,AO}	96.1^{VT,SP,AO}	2.94^{VT,SP,AO}
NBC11-04464	3.77^{VT,SP,C}	4.11^{VT,SP}	45.1^{VT,SP}	27.1^{VT,SP,AO}	96.5^{VT,SP,AO}	3.06^{VT,SP,AO}
Caracas	3.64	3.44	69.7	36.1	121.8	4.97
AG-Outback	3.00	3.13	51.9	26.1	99.9	3.00
VT Barrier	3.21	3.67	49.2	24.3	99.3	2.92
SP Banner	3.73	3.59	49.9	24.0	99.8	2.86
LSD	0.71	0.63	5.1	5.8	7.9	0.73